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INFARCTION IN THE HEART.

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I. Remarks on the Present State of Opinion concerning Infarction in the Heart.

THE closure of a large coronary artery is frequently a cause of sudden death, and if this peril be escaped, necrosis of the portion of the heart nourished by the closed artery may lead to dilatation, aneurism, or rupture, or may be followed by a scar. Yet closure is not always mortal. At times the pathologist finds the heart wall healthy in the area once supplied by an artery evidently long diseased and probably long occluded. The necrosis which should have followed is not there. Frequently indeed the arteries are so narrow as to be obviously incapable of feeding their vascular areas with the full streams which the laborious heart demands. Many questions are raised by these extraordinary phenomena. Whence comes the blood that keeps the part in life after its normal

arterial supply has failed? Do the coronary arteries anastomose? Is each portion of the heart wall traversed by branches from several arteries, or does each artery feed a district exclusively its own? Of what assistance are the coronary veins and the vessels of Thebesius? How long does the ischæmic part remain contractile? These and many similar problems confront us.

One of these problems has been much studied. Attention has long been paid to the possibility of anastomosis of the coronary arteries. The description of these vessels accepted for many years declared that the right and left coronary arteries anastomose freely and that this anastomosis is particularly rich at two points, — the junction of the posterior interventricular with the auriculo-ventricular grooves, and the junction of the anterior and posterior ventricular grooves at the apex of the heart. This view is said to have been first expressed by Ruysch.[1] It was upheld by the anatomists of the early part of the eighteenth century. In the middle of that century the accuracy of the description began to be questioned. The true condition was stated by Senac. The main coronary arteries anastomose only through the smallest arteries and through capillaries, and the communication is so slight that the closure of an artery puts a stop to the circulation in the part which it supplied. A collateral supply through communicating branches is not established. Great names support this description. Thus Henle[2] writes that Hyrtl's[3] statement that no anastomosis takes place between the larger branches of the right and left arteria coronaria is easily confirmed by injecting the two arteries with injection masses of different colors (p. 87). Moreover, observations have been made on the living animal. Cohnheim and v. Schulthess-Rechberg[4] severed the coronary artery in dogs and observed that little or no blood escaped from the distal end of the severed trunk. Infarctions have been produced experimentally by Kolster[5] and by Porter.[6] They are the invariable consequence of the ligation of coronary arteries in the dog. Notwithstanding these and other concordant observations, occasional

[1] Ruysch: quoted by Dragneff: Bibliographie anatomique, 1896, iv, p. 114.

[2] Henle: Handbuch der Anatomie des Menschen, Gefässlehre, 1868, pp. 85–87.

[3] Hyrtl: Lehrbuch der Anatomie des Menschen, 1889, pp. 1025–1027.

[4] Cohnheim and v. Schulthess-Rechberg: Archiv f. pathol. Anatomie, 1881, lxxxv, p. 510. Also Porter: This journal, 1898, i, p. 155.

[5] Kolster: Skandinavisches Archiv für Physiologie, 1893, iv, p. 1.

[6] Porter: Archiv f. d. ges. Physiol., 1893, lv. p. 366.

authors continue to speak for rich anastomosis, and the erroneous idea of the "two circles" still exerts an unfortunate influence.

The causes of this regrettable conflict over a question of much practical importance are to be found in an inexact conception of the nature of "terminal" arteries, and in imperfect knowledge of the ways in which the heart muscle in the area of an occluded artery may be saved from starvation. It has too often been assumed that the coronary arteries are not terminal because fine injections into one artery can be made to pass into other arteries.[1] It is just this assumption that reveals the obscurity in many minds. It is quite true that fine injections can be made to pass from one coronary artery into another. But this proves nothing except that the arteries communicate, and communication has never been denied. It is the kind of communication which is important. The idea of terminal arteries is physiological, not anatomical. They differ from other arteries in that the peripheral resistance in the anastomosing vessels is too high to be overcome by the blood pressure in any of the arteries of which the communicating vessels are branches. The rapid closure of the terminal artery cuts off the nutrition of its capillary area because sufficient blood for the life of the area cannot be sent through the communicating vessels on account of the high resistance in them. The resistance in the communicating vessels and not merely their size is the determining factor. Artificial injections on dead or "surviving" hearts cannot imitate the intricate complex which determines the resistance to the passage of normal blood through normal vessels.

It has been thought by some that an adequate arterial anastomosis is the only possible explanation of the survival of areas the arteries of which are occluded. Sometimes an anatomosis is found. Dragneff,[2] in a recent study of a series of sixteen human hearts, found an anastomosis in two cases, that is, in thirteen per cent. When the occluded artery happens to be one of the anastomosing exceptions, the explanation of the survival of its area is easy. These rare coincidences the pathologist often has generalized. Where no infarction occurs and no anastomosis is found, it is often thought that arterial anastomosis nevertheless is present, but has been overlooked. Another explanation of survival is furnished by the recent investigation of Pratt,[3] who has demonstrated that the mammalian heart will beat for

[1] MICHAELIS: Zeitschrift für klin. Medicin, 1894. xxiv. p. 270.

[2] DRAGNEFF: Bibliographie anatomique, 1896, iv, p. 111.

[3] PRATT: This journal 1898, i, p. 86.

a very long time when fed at a pressure of an inch or two of blood through the veins of Thebesius or the coronary veins. Evidently this method of nutrition may be of the first importance to the life of parts, the arteries of which are not too rapidly closed. Further, it has been demonstrated that in some situations purely capillary anastomosis may suffice for an adequate collateral circulation, and under certain conditions, as when the circulation is vigorous and the occlusion brought about slowly, this possibly may be true of the heart. Finally, it should be remarked also that we are yet in ignorance of the rôle which may be taken by lymphatics in the rescue of areas the arteries of which are very slowly occluded.

It is evident from these reflections that neither the passage of fine injections from one coronary artery to another, nor the occasional presence of arterial anastomosis, nor the rare survival of parts, the supplying artery of which is wholly or almost wholly closed, weighs in the balance against the facts that the distal stumps of severed coronary arteries bleed little or not at all,[1] that in the human heart the areas of closed arteries are almost always found to be infarcted, and that ligation of the coronary arteries in the dog's heart has thus far without exception been followed by the death of the part supplied. The coronary arteries are certainly terminal, with rare exceptions. The rarity of these exceptions should not be challenged by incautious statisticians. The fact that anastomosis may be present in thirteen per cent of the hearts examined, as in Dragneff's series, is true enough so far as the number of hearts is concerned, but has little real value. The chance of survival after closure depends on the percentage of arteries which anastomose — not on the percentage of hearts in which the anastomosis takes place. There are very many sufficiently large arteries in every heart. The chance that the artery occluded shall be one of those which happen to anastomose is extraordinarily small, and the weight to be attached to these rare cases in deciding the terminal nature of the arteries of the heart is therefore extraordinarily slight.

It follows further from the preceding considerations that the injection method is not well adapted to the study of the distribution of the coronary arteries. It is exposed to both objective and subjective errors. Objective errors are unavoidable. The distribution of the

[1] A few drops of blood may be squeezed out by the compression of the intramural vessels in the systole of the ventricle (PORTER: This journal, 1898, i, p. 155).

blood is determined during life by the peripheral resistance, and the peripheral resistance of the organ during injection is not that of the normal organ. The normal resistance depends on the inimitable arterial tonus. It depends further on the condition of the tunica intima, a highly sensitive structure, readily altered post mortem. Finally, it is dependent on the composition of the blood. Very slight changes in the blood are known to affect the flow through the vessels. The masses used for injection are widely different from the blood. The normal circulation through capillaries and the vessels next them in size is a function of the blood pressure in the smallest arteries. The exact amount of this pressure is not known, and probably never can be known, for it depends on a great number of interacting intricately balanced factors. Subjective errors are equally unavoidable. The observer who finds a communication must judge whether it was large enough in the normal state to so diminish the peripheral resistance that the normal blood pressure in the next larger artery could drive blood through it — obviously an impossible judgment. In short, the distribution of a terminal artery is a physiological question. The anatomist cannot determine it with certainty. The area which the terminal artery can keep alive is really the area which it supplies. This area is circumscribed by the points at which the resistance in the branches communicating between the artery and its neighbors becomes too great to be overcome by the blood pressure in those branches. The distribution of the coronary arteries should be mapped out by means of the infarctions which follow the ligation when the animal is kept alive, and by observations on human infarction.

It was for the purpose of mapping out the distribution of the coronary arteries by the infarction method that the present investigation was begun. It was resolved to study the problem also by means of injection with thin masses containing substances opaque to the Röntgen rays, — a method which avoids destructive dissections and corrosions, and which renders visible the vessels buried in the substance of the heart as well as those upon the surface. The overlapping or interlocking of areas, the character and especially the completeness of the infarction, the time at which the infarcted area ceases to be contractile, and the effect of infarction upon the sounds of the heart, were also studied.

The animals used were the cat and dog.

II. The Anatomical Distribution of the Coronary Arteries.

Henle's [1] description of the course of the coronary arteries in the human heart is as follows: "The two coronary arteries, of about equal diameter, arise from the aorta in the right and left sinuses of Valsalva, and proceed downward and outward for a few millimetres on the surface of the heart, between the auricular appendix and the conus arteriosus; each supplies a small branch to the conus arteriosus and the auricular septum. The right coronary artery turns to the right at the auriculo-ventricular groove, and passes between the right auricle and ventricle to the posterior surface of the heart, where its terminal transverse branch usually continues across the posterior interventricular furrow, and is distributed to the right half of the posterior wall of the left ventricle. A series of small branches is given off to the right auricle, and large branches are sent downward over the surface of the right ventricle; one of these descends at the right border of the heart, and another in the posterior interventricular groove to the apex. The left coronary artery divides at the auriculo-ventricular groove into two branches, one descending in or near the anterior interventricular groove, while the other turns to the left to continue between the left auricle and ventricle to the posterior surface of the left ventricle. The descending ramus distributes branches toward the left to the anterior wall of the left ventricle and several branches inward to the interventricular septum. The transverse branch supplies the left auricle and the left border of the left ventricle, its size and distribution varying with the size and distribution of the transverse branch of the right coronary artery. The larger of these usually supplies a branch to the upper posterior half of the interventricular septum."

In order to determine accurately the differences in detail between the distribution of the coronary arteries in man and in the cat and dog, injections of the coronary arteries were made with a mass consisting of equal parts of starch and bismuth subnitrate, rubbed up with water to the consistency of thin cream. The heart was then examined under the Röntgen rays with the fluorescope and finally radiographed by laying it, whole or appropriately divided, on dry plates specially prepared and exposing it a sufficient time to the Röntgen rays. In successful injections this procedure gave the dis-

[1] Henle: Gefässlehre, 1868, p. 85.

tribution of very small arteries (0.2 to 0.1 millimetre in diameter). It has already been pointed out that this method avoids destructive dissections and corrosions — the tissues remain intact.

The coronary arteries in the cat's heart. — The two main coronary arteries of the cat's heart arise from the aorta in the manner already described for the human heart. The left is always the larger of the two, and has a wider distribution. It supplies the whole of the left ventricle and auricle, the greater part of the interventricular septum, and a portion of the lower posterior wall of the right ventricle, upon which it often encroaches further. From the aorta it passes downward to the left and bifurcates just before reaching the auriculo-ventricular groove into two branches of unequal size. The smaller branch (ramus descendens) passes obliquely downward in the anterior interventricular groove to the apex of the heart. In its course it gives off to the left four or five large branches which ramify over the surface of the anterior wall of the left ventricle. Two or three small branches are given off to the right, but do not pass on to the right ventricle. Of the deep branches the most important is the artery of the septum, which arises about one millimetre below the bifurcation of the main trunk and passes downward and backward into the ventricular septum, at first close to the upper border of the septum, then curving downward along the posterior margin to its lower angle. The other deep branches of the descendens are small, and supply the anterior portion of the septum towards the apex.

The larger branch of the left coronary artery (ramus circumflexus) curves backward in the auriculo-ventricular groove around the left border of the heart. Arriving at the posterior interventricular groove, it bends downward at a right angle and proceeds along the groove to the apex of the heart. The main stem rarely encroaches upon the right ventricle. Near the apex, however, and sometimes nearer the base, superficial branches are given to the right ventricle. A large branch — at the left border of the heart — and many smaller branches are given to the left ventricle. The left auricle receives two large and several smaller branches. One of the two larger branches arises close to the origin of the circumflex, proceeds upward over the anterior surface of the auricle between the auricular appendix and the aorta, and ramifies over the superior surface of the auricle; the other is given off on the posterior surface and passes upward to the left of the pulmonary veins, where it divides, one branch going to the appendix and upper surface of the auricle, the other to the right

— sometimes reaching the right auricle. In the posterior interventricular groove, the descending stem gives off a number of branches to the upper and posterior margins of the septum.

The right coronary artery passes almost horizontally through a mass of fat into the auriculo-ventricular groove, and winds backward around the right border of the heart to or near the posterior interventricular groove. Here the artery, now very small, often turns abruptly and passes downward and forward for a short distance in the wall of the right ventricle. At the right border of the heart it gives off a large vessel which supplies the upper three fourths or more of the right ventricle. Near the aorta a short branch passes to the conus arteriosus. The auricles receive branches similar to the auricular vessels derived from the ramus circumflexus of the left coronary artery; the posterior one of these passes to the right side of the inferior vena cava and sends a branch toward the left auricle, between the two cavæ. The right coronary artery also supplies the upper part (basal portion) of the septum, the size and number of the vessels depending on the relative size of the ramus circumflexus sinister. No artery was found in the interauricular septum.

The coronary arteries in the dog's heart. — The distribution of the coronary arteries in the dog and cat differ (1) in that in the dog the artery of the septum usually arises independently from the left coronary artery, close to the origin of the ramus descendens and ramus circumflexus. The arteria septi passes closer to the anterior margin of the septum than in the cat, and supplies less of the upper posterior portion, which is left mainly to branches of the circumflexus. (2) The descendens gives off just below the middle of its course an important branch, which passes obliquely to the left and supplies the lower third of the left ventricle. (3) The terminal portion of the descendens usually supplies more of the apex than in the cat. (4) The branches passing from the descendens to the right are larger; they encroach somewhat upon the surface of the right ventricle. The deep branches are few and small.

The anastomosis of the coronary arteries studied with the Röntgen rays. — The question of anastomosis receives considerable light from the method of examination with the Röntgen rays, which obviates the disturbance and destruction of small vessels which must occur in dissections or corrosions. Eight cat and eight dog hearts were injected with the starch-bismuth mass and examined with the fluorescope

and by radiography. Anastomoses between vessels 0.1 millimetre in diameter were not infrequent, though not numerous. Vessels smaller than 0.1 millimetre were not injected by the mass employed. Only in one case was there found an anastomosis which could be supposed effective. This instance of free communication occurred in a dog heart in the posterior wall of the right auricle, between posterior auricular branches about 0.7 millimetre in diameter derived from the right coronary and circumflex arteries. The right coronary artery in this dog was completely injected from the circumflex branch of the left. This case is peculiarly instructive for the reason that although the injection mass passed through the anastomosis with great ease, the anastomosis had failed to re-establish the circulation through the right coronary artery, which had been ligated thirty-six hours before, as will be detailed in a subsequent paragraph. It may be well to note that the single anastomosis which Cohnheim found in a large number of injections of the dog's heart, as well as one of the two which Dragneff found in his injections of sixteen human hearts, were also between the posterior auricular branches of the right coronary and circumflex arteries.

III. The Physiological Distribution of the Coronary Arteries.

I purpose now to determine the areas within which the arteries of the heart perform their physiological function, — the areas which they normally supply with blood, and which accordingly depend on them for normal nutrition. Evidence has been presented to show that the coronary arteries are beyond question terminal. The ligation of a terminal artery deprives the part to which it is distributed of blood, gives over its charge to coagulation necrosis, and thus maps out the area in which its physiological function is performed.

The arteries ligated were the circumflex, descending, and septal branches of the left coronary artery, and the main trunk of the right coronary artery. In the cat the circumflex was ligated three times, the ramus descendens eight times, and the right coronary artery once. In the dog, the circumflex was ligated once, the descendens four times, the arteria septi three times, and the right coronary artery once.

The infarcts produced by the ligation of the right coronary or of one of the main branches of the left coronary artery in different animals of the same species are in the main identical in extent.

Method of operating. — All the operations were performed with strict asepsis. In operating on cats, the animal was anæsthetized with ether, tracheotomized, and an incision extending from the second to the sixth rib of the left side made through the skin and the pectoral muscles, close to the margin of the sternum. To prevent bleeding from the intercostal arteries sterilized ligatures were passed around the third, fourth, and fifth costal cartilages near the sternum and again near the outer margin of the wound, the ends being left long, so that they might be used later to draw the severed ends of the cartilages together. The three cartilages were now divided an inch to the left of the sternum. With the opening of the thorax, artificial respiration was begun. The parietal pericardium was freed from fat sufficiently to permit an incision one to two centimetres long, parallel to the left margin of the conus arteriosus. The visceral pericardium was then torn slightly with forceps near the course of the artery to be ligated, the artery laid bare with a blunt seeker, and a ligature passed around it with a small curved threaded hook bent at right angles to the shaft. No attempt was made to close the incision in the pericardium. The ends of the divided costal cartilages were brought together by means of the long ends of the ligatures which had been passed around them. The external wound was sewed up and dressed with a layer of corrosive sublimate gauze and a thick pad of cotton. After the animal had begun independent respiration, the tracheal cannula was removed and the wound in the neck closed. Finally, the chest and neck were thoroughly bandaged.

The procedure in the case of dogs was not essentially different. Dogs received from one to three centigrams of morphine sulphate half an hour before etherization. The dose was repeated from two to four hours afterward to prevent restlessness. The operation outlined above was modified in that the second rib was also divided, and incisions two to three inches in length were made in the first and fifth left intercostal spaces so that the wall of the thorax could be drawn aside and the opening into the chest enlarged.

In several dogs the arteria septi was ligated.[1] As the difficulty of this operation has been commented on by Professor Kronecker[2] it may be stated that special precautions are needed to secure its success. There should be four assistants, one to blow air into the lungs through a two-necked Wolff's bottle containing ether, a second to

[1] These operations were done by Dr. Porter.

[2] KRONECKER: Zeitschrift fur Biologie, 1896, xxxiv, p. 554.

hold the lips of the wound apart with heavy retractors, a third to assist with the instruments, and a fourth to hold an electric lamp in such a way that the operator can catch the light upon a frontal mirror of the sort used by laryngoscopists. This mirror is nearly indispensable. It should be turned up against the forehead so that both eyes may be uncovered. With this light the slight differences in color between muscle fibres and small arteries are much more easily made out. The heart is laid bare as described above. The edges of the pericardium are sewed to the chest wall with two stitches on each side — thus raising the heart. The operator seizes with dissecting forceps the fat and connective tissue at the base of the aorta near the origin of the left coronary artery, and with a second dissecting forceps removes the tissues over the junction of the ramus circumflexus and ramus descendens. A blunt flattened seeker is then employed to press back the muscle fibres and other tissues about the junction, burrowing under until the arteria septi comes into view. If the latter arise from the right anterior aspect of the main trunk or the ramus descendens, it may be found easily; if from the left posterior aspect, the search will be long and trying; in such case the ramus descendens may be raised for a few seconds — not longer — upon a flattened bent hook. Ligation is accomplished by passing a threaded slightly curved aneurism hook beneath the ramus septi, releasing the handle, grasping the end of the thread with dissecting forceps and drawing back the aneurism hook. In the preparation of the artery no blood whatever must be lost; a few drops will so stain the tissues as to render success very doubtful. The assistants must be thoroughly trained, and the operation must be done rapidly and without a flaw. It is particularly necessary that the bellows be managed skilfully.

The after treatment of the animal is nearly as important to recovery as the manner of operating. Cats should be placed in a small box upon clean straw and kept warm until the immediate effects of the operation have passed away. Dogs should be laid on the side upon the dog-board and supported on comfortable cushions the covers of which are made of glazed stuff that can be wiped clean. The fore-limbs are held near the board by one short tether, the hind limbs by another. A leather strap passes over the chest, and a second strap over the neck, both too loose to press upon the dog, but tight enough to prevent his slipping off the board or raising the head. These precautions lessen the risk of heart failure. Morphia should be given

in very small quantities to prevent restlessness during the first day. The care of the animal should be that received by a serious case in a hospital ward.

Infarcts in the cat's heart. — The extent of the infarct following ligation of the ramus descendens in eight cats was almost the same in each case. Variations were far slighter than in the infarcts following ligation of either of the other arteries. The infarct of the descendens includes the whole of the anterior wall of the left ventricle from the anterior interventricular furrow to the left border of the heart, except a small triangle in the upper left corner of the surface. It also includes the anterior portion of the apex of the heart, the anterior two thirds of the septum, and the anterior papillary muscle. The descendens area thus forms a quadrilateral, one angle of which is at the point of ligation. The infarct does not extend above the auriculo-ventricular groove, nor does it involve the whole apex of the ventricle, nor the posterior third and upper border of the septum. On section, the infarct is seen to include the whole thickness of the anterior wall of the left ventricle and the left papillary muscle. But in the septum only the left half, the side turned towards the left ventricle, is infarcted.

The infarct following the ligation of the circumflex artery involves a much wider area. It extends over the posterior wall of the left ventricle and auricle, from the apex of the heart to the point of entrance of the pulmonary veins into the left auricle. At the upper end of the posterior interventricular groove a small triangular space on the left ventricle remains untouched. The posterior papillary muscle, the posterior third of the septum, and a variable extent of the surface of the right ventricle are also infarcted. In the left auricle, the posterior wall up to the superior border, and the posterior border of the appendix are infarcted. The pulmonary veins show no evidence of infarction. On section, it is observed that the posterior wall of the left ventricle and auricle, and the posterior papillary muscle, are infarcted through their entire thickness, whereas in the septum the posterior third is usually involved only on the side facing the left ventricle, and in the case of the right ventricle only the pericardial surface is attacked.

The infarct produced by ligation of the right coronary artery occupies the territory between the descendens and the circumflex area, namely, the whole thickness of the greater part of the wall of the right ventricle, the whole thickness of the posterior wall of

the right auricle, and the posterior portion of its appendix, and finally the right ventricular aspect of the posterior third of the septum. The circumflex and right coronary areas commonly overlap to a variable extent on the posterior surface of the right ventricle; in these cases, the right coronary infarct involves the endocardial portion of the wall of the ventricle, while the circumflex infarct occupies the pericardial portion, as has already been mentioned.

In these several ligations the auricular appendices were not completely infarcted because the nutrient branches to their anterior wall arise close to the origin of the main trunks and were therefore not included in the ligatures.

The inferior vena cava remained uninvolved.

Infarcts in the dog's heart. — In the heart of the dog the areas of infarction following ligation of the circumflex and the right coronary arteries are similar to those of the same arteries in the cat. The infarct of the circumflex exhibits some variation; in two cases it involved the whole apex of the heart. The chief points of difference between the two animals relate to the descending branch of the left coronary artery. In the dog, the artery of the septum is given off independently of the descendens, or in rare instances very close to its origin, so that the arteria septi is not included in the ligation of the descendens. In the dog the descendens infarction is therefore limited chiefly to the anterior wall of the left ventricle and the inferior (apical) anterior portion of the septum. To a slight extent, but relatively further than in the cat's heart, it encroaches on the right ventricle. The infarct produced by ligation of the septal artery is triangular in shape, with the apex of the triangle near the ligature. Whether the auricular septum was involved in this infarct was not observed.

The infarction in a dog in which the descendens was ligated by W. T. Porter in 1893[1] will be of interest here. This dog lived four days. Post mortem it was found that the artery was tied six millimetres from its origin. A thrombus filled the artery on the proximal side of the ligature. The interventricular septum, which was about 45 mm. broad and 6 mm. thick, contained a white wedge-shaped infarct. Seven millimetres below the ligature (apical direction) the infarct measured 13 mm., and 25 mm. below the ligature 23 mm. from posterior to anterior margin. A similar wedge occupied the anterior wall of the left ventricle, extending outwards from the

[1] Porter: Arch. f. d. ges. Physiol., 1893, lv, p. 367.

interventricular furrow. The apex of this part of the infarct lay near the ligature. Thirty millimetres below the ligature (apical direction) its breadth was nearly 30 mm. The infarct was plainly visible on the inner surface of the ventricle. It comprised the whole thickness of the ventricular wall.

One case in my series of infarcts in the dog's heart deserves particular attention. In this case the right coronary artery was ligated, and the animal was killed thirty-six hours later. As the infarct did not seem to be well marked on the pericardial surface, the circumflex artery was injected in order to determine the existence of an anastomosis. This injection succeeded without difficulty in filling the trunk and all the ramifications of the right coronary artery, and demonstrated a large, well-defined anastomosis 0.7 mm. in diameter in the posterior auricular wall. The infarct proved to be complete, the macroscopical appearance being corroborated by microscopical examination. No better illustration could be desired of the extent to which the arterial tonus controls the peripheral resistance and thus determines within wide limits whether a communicating branch shall during life be wide or narrow — an open or a closed gate for collateral circulation.

Variations in the areas of infarction. — Variations in the boundaries of infarcts are particularly marked in the infarcts of the circumflex and right coronary arteries. The descendens shows a very constant distribution, which in the cat varies only at the posterior border of the septum. The greatest variation in the area of any artery takes place in the distribution of the circumflex. The left margin, that adjoining the descendens, is uniform; its septal margin, however, may involve the whole posterior border of the interventricular septum and a portion of the right ventricular wall, or may be sharply limited by the posterior interventricular groove.

The margins of cardiac infarcts are not clear-cut and sharply defined, but quite irregular. They contain patches of apparently normal tissue, and are bordered by small infarcts scattered through the adjacent normal heart wall. This will be further discussed in the report of the histological details.

Comparison of the results of the physiological with those of the anatomical method. — It will be seen that the physiological distribution of the coronary arteries, obtained by the method of infarcts, corresponds in the main with the distribution outlined by anatomical methods. The physiological method is certain — the anatomical at best conjectural.

The physiological method defines accurately the relation of adjacent arteries, and the part which each plays in the nutrition of the portion of heart wall to which both are distributed. It demonstrates that two arteries may supply parts of the same area and still be independent of each other. Overlapping of this sort occurs particularly between the circumflex and the right coronary arteries in the posterior wall of the heart, and between both these vessels and the arteria septi in the posterior third of the septum.

The overlapping of the distribution of main stems and larger branches must be carefully distinguished from that of smaller branches. In the case of smaller branches, as has been shown by Kolster,[1] the area of infarction is not homogeneous, but consists of small foci of necrosis separated by masses of normal tissue. A method of distribution identical with this occurs at the margins of the larger infarcts, while the body of the infarct contains only completely necrotic material and no interspersed normal tissue.

IV. The Contractility of the Anæmic Area.

Many observers have noted that the beat of the heart continues for a brief time after the closure of large branches of the coronary arteries, but no one has studied as yet the contractility during the first stages of infarction. The discovery that portions of the mammalian ventricle will resume their contractions if fed with defibrinated blood enables us to determine how long an ischæmic area in the heart remains contractile. A coronary artery must first be ligated, the animal kept alive until infarction has begun, and the ischæmic portion then cut out and fed with defibrinated blood through a branch of the coronary artery.

Experiment April 18, 1898. The descending branch of the left coronary artery in a dog was ligated in the manner already described. After an interval of six hours the dog was bled from the carotid artery, and the blood defibrinated, diluted with 0.8 per cent sodium chloride solution, and placed in a reservoir under pressure, in a warm bath heated to 37° C., according to the method described by Porter.[2] The heart was then extirpated. The area of distribution of the descendens was found to be pale grayish-brown, translucent, and sharply marked off from the contiguous normal tissue. A cannula was

[1] Kolster: Skandinavisches Archiv für Physiologie, 1893, iv, p. 25.

[2] Porter: Journal of experimental medicine, 1897, ii, p. 391, and This journal, 1898, i, p. 514. It is to be remarked that dilution with saline solution is a convenience — not a necessity. Undiluted blood will serve.

tied into the descendens below the ligature, and the anæmic area excised, care being taken not to include any of the normal tissue. The cannula was then connected with the reservoir of defibrinated blood and the piece of anæmic heart muscle perfused, under a pressure of 97 mm. Hg. The suspended muscle began to contract rhythmically within one minute after the blood stream had been established in it. Five minutes later it fibrillated, but after its blood-supply had been interrupted for several minutes, resumed its rhythmical contractions, and continued to beat regularly at 104 contractions per minute for one hour, when it was disconnected from its blood-supply.

Experiment May 2, 1898. The same preliminary steps were taken as in the foregoing experiment. An interval of eleven hours was allowed to pass after the ligation of the artery. The dog was then bled as before and the heart removed. The anæmic area was opaque, distinctly yellowish in color, uniformly and sharply outlined. When, however, perfusion was begun, under a pressure of 60 mm. Hg., the excised anæmic muscle responded immediately with rhythmical contractions, weak at first, then growing stronger, and these continued uninterruptedly for thirty minutes. The contractions were markedly weaker in the centre of the area and increased in strength toward the periphery. The weaker contraction in the peripheral parts was evident also when the piece of muscle was cut into longitudinal strips, each strip including a branch of the nutrient artery. The strips from the peripheral portion of the area contracted more vigorously than the central ones. The precaution was taken in this experiment, as in the preceding one, to include in the preparation only the anæmic area.

From these experiments it will be seen that the part of the heart wall rendered anæmic by the interruption of its arterial blood-supply remains contractile at least eleven hours. How much longer contractility may persist has not been determined. The most recent infarct examined microscopically was twenty-two hours old, and its appearance indicated that contractility had been completely lost.

It has thus been demonstrated that (1) the heart muscle continues to contract for a time after the interruption of its circulation, and (2) it retains its ability to contract for a much longer period; whether it actually does contract for longer periods has not been determined. The contractions of the anæmic area may be accounted for in two ways: the energy for the contraction is either derived from some store of contractile material present in the muscle at the time the nutrient artery is ligated, or is supplied after ligation by the establishment of a feeble, temporary circulation, which becomes progressively less efficient and finally fails to prevent the death of the area. The sources from which this circulation may be derived

are three; namely, the vessels of Thebesius, the coronary veins, and possibly to a slight extent the adjacent capillary systems. Of these the first two present conditions peculiar to the heart, and require discussion.

The coronary veins have a free anastomosis with each other, and, as Pratt [1] has shown, a direct communication with the vessels of Thebesius by vessels larger than capillaries. Both the coronary veins and the vessels of Thebesius are practically valveless. It may be assumed therefore that in them blood will flow in the direction of least resistance, whether with or opposite to the normal direction. In an inquiry into the influence of the heart-beat on the flow of blood through the walls of the heart Porter [2] has shown that each contraction compresses the intramural vessels. The force of this compression is very considerable. If the apical half of the ventricles of the dog's heart is removed and fed at a constant pressure with defibrinated blood through a cannula tied into the ramus descendens, blood from the severed arteries in the margins of the preparation will shoot far out with each contraction. This systolic compression of the intramural vessels is very important in our present problem. It is known that the anæmic area continues to contract for a time. The arterial supply being shut off, the systolic compression of the vessels within the wall in the anæmic area will empty them of blood. At the beginning of diastole these empty patulous vessels, in which the blood-pressure has been reduced to nothing or less than nothing, will be readily filled from the superficial coronary veins and the vessels of Thebesius. The former lie upon the surface — they are extramural — and are not emptied by the systolic squeeze; the latter open into all the cavities of the heart, and may draw blood directly from an always ready source. The quantities of blood which may be thus secured are relatively small but may have important effects. Magrath and Kennedy [3] have proved that the mammalian heart will beat on a surprisingly small supply, and reference has already been made to the fact that the mammalian heart can be kept beating by the blood drawn from either the vessels of Thebesius or the coronary veins. Finally, we are not without direct experimental evidence that the sources just discussed are of value. I find, as has already been mentioned, that the contractility of the anæmic area is least in the centre and greatest at the mar-

[1] Pratt: This journal, 1898, i, p. 92. [2] Porter: *ibid.*, p. 145.

[3] Magrath and Kennedy: Journal of experimental medicine, 1897, ii, p. 13.

gins, which are more favorably placed than the central parts for receiving blood in the manner suggested. When a coronary artery is closed suddenly, these auxiliary sources never succeed in preventing coagulation necrosis, but it must be conceded that their value may be great in the many instances in which closure extends over months and years.

V. Gross and Microscopical Changes in the Infarcts.

Kolster [1] has described the changes which take place when a small branch of a coronary artery of the heart is ligated. As a greater area and other relations are involved in the infarcts produced in the present investigation, it has been deemed necessary to study again the gross and microscopical changes in the area of infarction.

Histological method. — The cats operated upon were killed at periods of thirty hours, three, four, five, six, and fourteen days, two months, and seven months after the ligation; in dog hearts the intervals were twenty-two, thirty, and thirty-six hours, and ten, fifteen, and ninety days. A view of the progressive changes in the infarcts was thus obtained. The infarcted cat hearts were hardened *in toto* in Zenker's fluid forty-eight hours, washed for the same period in running water, and then rehardened in rising grades of alcohol, during which process they were passed through a seventy per cent solution of iodine. They were then imbedded in celloidin and each block was hardened first by moderate evaporation and then in eighty per cent alcohol. The blocks were mounted whole, and transverse sections varying from eighteen to thirty micromillimetres in thickness were cut from the apex toward the base. The sections were preserved in series, those of each millimetre being kept separately. Of these, one from each millimetre was stained and mounted, so that a series with intervals of one millimetre was obtained from each heart. Alum-hæmatoxylin and a one per cent aqueous solution of eosin were used as stains. Selected portions of infarcts in the dog's heart were treated in the same way, but no serial sections were made.

To determine the presence of fatty changes small pieces of infarct were hardened in Flemming's solution.

Gross changes. — Macroscopically the infarcted area becomes white or whitish yellow in color, opaque, and flaccid. On section it presents a smooth, bright surface. In three cases, one in the cat's heart and

[1] Kolster: Skandinavisches Archiv für Physiologie, 1893, iv, p. 1.

two in the dog's heart, the infarct was hæmorrhagic. In all cases the portion of the heart wall involved was dilated to a greater or less extent; the dilatation increases up to the fourth day. In all recent cases the veins arising in the infarcted area and their anastomosing branches remained intact; after the fifth day their presence was not noted in any case. Infarcts two weeks or more old became whiter, began to contract, and showed all the changes of newly developed connective tissue. In two cases polypous growths were present in the pericardium over the infarcted area. In the oldest case in the series (seven months) the infarct had been slightly reduced in size and had been converted into a thin, white, semi-transparent, glistening membrane, into which the muscular wall inserted as into a tendon. Neither in this case, nor in any other in the series, was the heart wall sufficiently dilated to form an aneurism. No clots or visible fibrinous sheets were found on the endocardium.

Histological changes in recent infarcts. — The microscopical examination sought to establish the character of the degeneration in the infarct, and the progressive changes which take place as the interval after ligation increases, and to determine whether degeneration was complete throughout the area or whether patches of tissue remained normal here and there.

Coagulation necrosis is found fully developed through the whole area of the infarct as early as twenty-two hours after ligation of the artery (dog, circumflex). At this hour many nuclei can no longer be stained, some show irregularities and fragmentation, and a few are swollen and vacuolated. In the earliest cases examined, the protoplasm had undergone granular degeneration, but in cases three or four days old hyaline degeneration is more frequent. Small vacuoles occur in many muscle cells, usually several in each cell so affected. In sections of pieces of infarct hardened in Flemming's solution, small black granules are found in some of the muscle cells. They are not present in many cells, but are very characteristic where they occur. The endocardial connective tissue, and the muscle cells bordering immediately upon it, are in some cases not involved in the general necrosis. The endocardium in these is normal; in other instances, however, it is covered with a thin membrane of fibrin, which encloses a few red corpuscles. At the margins of the infarct, and only there, groups of normal muscle fibres are scattered through the necrotic tissue, and sometimes acquire macroscopical size. These interruptions of necrotic area by normal tissue are identical with the infarcts which

Kolster produced by the ligation of a branch of the ramus descendens.

The condition of the principal blood vessels and the capillaries in the infarcted area varies greatly. Throughout the larger part the capillaries and small vessels contain no blood corpuscles and are difficult to distinguish. At the margins of the infarct they are usually distended with blood, and small extravasations of red blood corpuscles are sometimes present in the surrounding tissue. The vessels near the endocardium frequently share in the obliteration which has overtaken those in the greater part of the infarct. In some cases, however, some of these vessels are distended, and in one or two cases small hæmorrhages were observed in the centre of the infarct near the endocardium. The veins on the pericardial surface are always filled with blood.

In very recent cases the connective tissue in the centre of the infarct does not seem to be involved in the necrosis. Its nuclei retain their power of staining and are neither fragmented nor irregular. In older infarcts (three to five days) these have undergone degeneration.

Changes in the infarct with progressing age. — Infarcts three days old are little changed from the condition on the first day. Hyaline degeneration is more frequent, and cross striation less pronounced in the muscle fibres which retain their form than in more recent infarcts, and the connective tissue has in many cases become involved in the necrosis. The periphery of the infarct has become infiltrated with leucocytes, and the connective tissue cells both within the margin and just outside the infarct have begun to proliferate and extend slightly into it. The further changes up to about the sixth day consist in the steady penetration of leucocytes and newly formed connective tissue cells into the infarct, with absorption of portions of necrotic tissue. By the fourteenth day bands of connective tissue cells traverse the whole infarct, carrying capillaries with them. The mass of necrotic muscular tissue has almost entirely disappeared; connective tissue fibres have begun to be formed. Extravasated red blood corpuscles retain their outline even up to this time, and seem to be absorbed less easily than the necrotic muscular tissue. The newformed connective tissue is not confined to the limits of the infarct, but sends large strands into the normal muscular tissue, displacing some of it, and enclosing and isolating other portions.

It must be noted here that in almost all cases the larger vessels,

both arteries and veins, are still intact, that is, their cells exhibit no alterations in the protoplasm or in the staining power of the nuclei.

The next stage examined was an infarct of two months, which was produced by the ligation of the ramus descendens. It did not give the results which might have been expected to follow the conditions noted in the earlier stages. Macroscopically, a thin, soft, narrow white band of scar tissue was found parallel to the anterior interventricular groove and a little to the left of it. Microscopically this consisted of a thin wall of muscular tissue, apparently normal, traversed from side to side by bands of connective tissue, which, however, nowhere formed the entire heart wall. In the septum the anterior third was replaced by a wedge-shaped mass composed of a firm, close-woven white areolar tissue, affording conclusive evidence that the ligature was successful. Whether the small extent of the anterior wall of the left ventricle which had become fibrous was due to anastomosis in that part or to regeneration of muscle remains a question. There is, however, no evidence for regeneration.

The histological changes in infarcts of the dog's heart are identical in character with those in the heart of the cat, and progress with equal rapidity. Local hæmorrhagic infiltration occurred more frequently in the dog than in the cat. In one case the infiltration took place in two narrow zones parallel to the endocardium. One of these lay just within the muscular layer, next the endocardial connective tissue; and the other, of about equal width, extended along the middle of the heart wall.

VI. Changes in the Heart's Action following Ligation.

The changes in the force and frequency of the heart-beat immediately following the ligation of a coronary artery were described by Porter in 1894.[1] In the present investigation, in a series of twenty-one cats operated upon, nine of which were not used in the foregoing portion of the investigation because they survived the operation only a few hours, the heart in thirteen gave no clear evidence of alteration in rate, in five showed a diminution in both force and frequency, and in three cases fibrillated; one of these recovered. In a series of nine dogs, the heart showed diminution in frequency of beat in five cases, but no change in force; there was no case of fibrillation. Accurate, extended observations of changes in force and frequency

[1] Porter: Journal of physiology, 1894, xv, p. 121.

would have interfered with the main purpose of the investigation by making it more difficult to keep the animal alive.

At a later period after the operation the pulse became more frequent and weaker; the rhythm sometimes remained regular, but in many cases was irregular and often intermittent. This condition usually persisted for thirty-six to fifty-four hours, and then gave place to a pulse normal in rate and rhythm.

Auscultation of the heart sounds was performed only in dogs, as the heart of the cat beats too rapidly to make this profitable. The sounds remained clear and distinct, and no murmurs could be detected. The pitch was unchanged, and no undue valvular quality could be made out in the first sound.

Death from sudden cardiac failure in cases which survived more than twenty-four hours occurred twice, each time after violent exertion. The first case was in a cat whose circumflex artery had been ligated five days before, and happened in a quarrel with another cat; the other case occurred in a dog in which the descendens had been tied fifteen days before; his death followed soon after he had been fed and had been jumping about to be petted.

Summary.

1. The coronary arteries are "terminal;" their anastomoses rarely permit the formation of a collateral circulation.

2. The physiological distribution of the coronary arteries obtained by the method of infarcts, corresponds in the main with the distribution outlined by anatomical methods.

3. The part of the heart wall rendered anæmic by the interruption of its arterial blood-supply may remain contractile at least eleven hours after the ligation of the artery.

4. The central part of the anæmic area undergoes coagulation necrosis more rapidly than the peripheral parts. Probably the outlying portions receive blood through the vessels of Thebesius and the coronary veins.

5. In animals which survived the ligation of a large coronary trunk thirty-six to fifty-four hours the pulse seemed normal in rate and rhythm and the heart-sounds were clear and distinct. No murmurs could be detected and no undue valvular quality could be made out in the first sound.

6. The results of the study of the anatomical distribution of the coronary arteries, the investigation of their anastomosis with the Rontgen rays, their physiological distribution determined by the boundaries of the infarction following their closure, and the gross and microscopical changes in the infarcts, cannot be stated in a brief summary, but must be sought in the text.

In conclusion I wish to express my indebtedness to Prof. W. T. Porter, at whose suggestion and under whose direction this investigation was carried on.

ON THE CAUSES OF THE ORDERLY PROGRESS OF THE PERISTALTIC MOVEMENTS IN THE ŒSOPHAGUS.

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IT is now generally assumed that the orderly progress of the peristalsis in the œsophagus is exclusively of central origin. This means that the first afferent impulse which is conveyed from the periphery to the centre of deglutition and which causes the co-ordinate contraction of the mylohyoid, pharyngeal, and laryngeal groups of muscles, travels further within the centre through several groups of ganglia, sends down successively along its route efferent impulses to the several divisions of the œsophagus, including the cardia, and causes hereby their successive contractions without the aid of new afferent stimuli. This view is based upon observations which demonstrate that the lower part of the œsophagus as well as the cardia continues to show the peristaltic contractions even after the ligation or division of the œsophagus. The observations upon the cardia were made by Kronecker and myself[1] and stand uncontradicted. The observations upon the œsophagus were made by A. Mosso,[2] and are diametrically opposed by the earlier experiments of Wild,[3] done in 1847 under the direction and special participation of Ludwig. Wild states in unmistakable terms that strong compression, ligation, or division of the œsophagus positively stops the progress of the peristalsis, and from his experiments he draws a conclusion just the opposite of ours, namely, that the peristalsis is caused by reflexes from the periphery, that is, from each part of the œsophagus itself. Mosso, who was stimulated to his researches on the peristalsis of the œsophagus by these very statements, reports simply his own experiments without offering any explanation of the strange contradiction between himself and Wild. So far as I know, this sub-

[1] KRONECKER and MELTZER: Archiv für Physiologie, 1883, Suppl. Bd., p. 328.
[2] MOSSO, A.: Untersuchungen zur Naturlehre, 1876, xi, p. 327.
[3] WILD: Zeitschrift für rationelle Medizin, 1847, p. 76.

ject has since Mosso not yet been reinvestigated, and although we ourselves have supported the view of Mosso, as harmonizing with our own, our experiments themselves were restricted to the cardia. A fresh study of this subject, which I have recently taken up again, makes it probable that the contradiction between Mosso and Wild exists rather in the conclusions drawn from the facts than between the facts themselves, inasmuch as the purpose and the method of these investigators were not exactly the same. Wild experimented upon the cervical œsophagus while Mosso studied the movements in the thoracic part; again, Wild studied the movements by direct observation, while Mosso judged whether peristalsis were present by the motion of a wire attached to an olive-shaped body which was introduced in the upper part of the thoracic œsophagus; finally, Mosso used ether for anæsthesia, while Wild injected opium in the jugular vein. With these points in my mind I started a series of experiments regarding the nature and the causes of the peristalsis in the œsophagus which have brought noteworthy results, some of which I shall now report briefly, illustrating them by the account of a few experiments.

I have in the first place repeated the experiments of Wild with the unimportant deviation that I used for anæsthesia morphine instead of opium. Here is an illustration.

Under local anæsthesia by cocaine the external jugular vein of a dog weighing nine kilos was exposed and 0.06 gram of morphine injected. This produced a fair anæsthesia, which lasted throughout the experiment. A cannula was inserted in the trachea and then the cervical œsophagus from pharynx to sternum exposed to full view. After each of the spontaneous deglutitions which occurred from time to time a well-defined peristaltic wave was seen passing down the entire visible length of the œsophagus. Mechanical stimulation by pressing the mylohyoid region or the larynx or by tickling the soft palate and pharynx with a rod introduced through the mouth failed to bring out any deglutition. However, by pouring water into the mouth assisted by pressure upon the mylohyoid region the animal could be made to swallow two or three times, the deglutitions usually following each other in rapid succession, and here the peristaltic wave was seen to sweep down the œsophagus only after the last swallow. A tight ligature was now put around the œsophagus at a distance of about six centimetres from the larynx, and the animal was made to swallow. The portion of the œsophagus above the ligature became distended immediately; there was no peristalsis below the ligature following this swallow. The animal, however, soon started without any new stimulus to swallow incessantly, and the upper portion of the œsophagus began to swell

to large proportions. Apparently there was a great effort, in which even the muscles of the neck seemed to take part, to force the swallowed mass through the ligature. The rapid swallowing and the entire forcing effort could be checked for a while by holding down the larynx, and from time to time there was also a spontaneous pause for a minute or two; but at no time could a peristaltic movement be discovered in the portion of the œsophagus below the ligature. As soon, however, as the ligature was opened the mass came down immediately the whole visible length of the œsophagus with a distinct peristaltic wave, without the occurrence of a new swallow. When the animal was now made to swallow by pouring water into the mouth, a peristaltic wave was again seen to run down the entire œsophagus after the last swallow. The ligating of the œsophagus and the opening of the ligature were repeated a number of times with invariably the same results. It is hardly necessary to state expressly that care was taken not to include the recurrent nerve in the ligature.

This experiment, then, brings out a simple confirmation of the statements of Wild with regard to the peristaltic movement in the cervical œsophagus.

In the following experiment in addition to the direct observation of the behavior of the cervical œsophagus, the method of Mosso was also employed to study simultaneously the action of the thoracic part of the œsophagus.

A dog of eight kilos was anæsthetized in the manner described in the previous experiment, the cervical œsophagus freely exposed, and a strong thread put loosely around it at a distance of six centimetres from the larynx. Below the thread a longitudinal incision was made in the œsophagus and an olive-shaped body of hard rubber introduced through it into the thoracic portion of the œsophagus. To this body was attached a long silk thread which passed over an elevated projection and was slightly stretched by a light weight, affording thus a means by which the movement of the olive-body could be easily observed. The introduction of the body caused no peristalsis of the thoracic œsophagus — the thread did not move. A spontaneous deglutition or a deglutition caused by water in the mouth was followed by a peristaltic wave in the œsophagus, but only to the longitudinal incision. Here some liquid escaped and no peristalsis was seen in the part of the cervical œsophagus below the incision nor did the thread move. The longitudinal incision was now closed by a few stitches, but so as still to permit the free movement of the silk thread attached to the olive-body. When now a peristaltic wave started in the œsophagus it promptly went down to the sternum, and soon the olive-body could also be noticed moving downward to the stomach. In this animal mechanical stimulation by a rod introduced into the mouth often caused

deglutition, but the latter was mostly restricted to the contractions of the mylohyoid, pharynx, and larynx, and rarely followed up by peristalsis in the œsophagus. By pouring water into the mouth, however, the characteristic ascent of the larynx was promptly followed by a distinct peristaltic wave in the œsophagus. The thread around the œsophagus was now tightened and the animal was made to swallow once. Immediately the entire complex of incessant swallowing, extreme distention of the part of the œsophagus above the ligature, and considerable efforts to force the passage, appeared on the scene in the manner described in the previous experiment; but here, too, at no time was there any peristaltic movement visible in the part of the cervical œsophagus below the ligature, nor was there any movement of the thread attached to the olive-shaped body. After loosening the ligature a wave immediately swept down the œsophagus, and the thread also indicated a downward movement of the olive-body. The tying and untying of the thread around the œsophagus was repeated a number of times with the same result. Finally a longitudinal incision was made in the upper part of the œsophagus. Now the peristalsis came down only to the incision, while the rest of the cervical œsophagus as well as the olive-body never moved. When now the œsophagus was ligated the swallowing did not produce any distention of the œsophagus, liquid and air escaped through the incision, and no incessant swallowing took place.

This experiment shows that when the œsophagus is ligated there is apparently neither in the cervical nor in the thoracic part a peristaltic movement, which would seem to be a direct contradiction of the statements of Mosso. In both these experiments, however, the animal was narcotized by morphine. To make the conditions of our experiments uniform with those in the experiments of Mosso we have conducted some of the experiments under ether anæsthesia. Following is an example.

A dog of about seven kilos was anæsthetized by ether in the usual way, then tracheotomy was performed, and ether given by tracheal cannula. The œsophagus was exposed and the olive-body introduced as in the previous experiment. The animal was now under such deep anæsthesia that water poured into the mouth remained there, and no stimulus would cause any deglutition. Anæsthesia was diminished, and the animal was allowed to come out of its influence gradually. The first effective stimuli brought out deglutitions which were not followed by any peristalsis in the œsophagus; next came a stage when deglutition caused by pouring water into the mouth was followed by peristalsis, but no peristalsis was seen after "empty" swallows brought out by mere mechanical stimulation. Finally a stage came in which the peristalsis took place promptly after every deglutition caused by any kind of stimulus.

The dog was now in a state of light anæsthesia. The œsophagus was now ligated and the lively swallowing and the swelling up of the upper part of the œsophagus appeared as in the previous experiments, but no peristalsis occurred below the ligature, nor did the silk thread indicate any motion of the olive-body. The experiments with ligation were repeated several times with the same results. Then the œsophagus was cut transversely at the middle of the neck; the lower end retracted into the thorax. When now the animal was made to swallow by pouring water into the mouth or by any other method, the silk thread regularly indicated the descent of the olive-body into the stomach about three to four seconds after each swallow, or after the last one, when deglutitions followed each other in rapid succession.

Here then is an experiment which also confirms the statements of Mosso. It should be added that in nearly all his experiments, Mosso tried to interrupt the progress of the peristalsis mainly by division of the œsophagus or by removing a part of it; the ligation he tried only in a single instance, but then he applied a method quite different from that which Wild and I have employed. I have reason to believe that this difference in the method may be of some importance to our question, but I am not prepared to discuss this point at present.

These few experiments teach us that the statements of Wild as well as of Mosso are both correct (and, what is more, even both conclusions seem to be correct), giving us at the same time the key of the apparent contradiction. We have seen that the appearance of the peristalsis in the œsophagus is considerably influenced by the degree of anæsthesia to which the animal is subjected.

At a certain deep degree of anæsthesia no peristalsis follows the first act of deglutition.

Then comes a stage when the peristalsis appears in the œsophagus only after such deglutitions as are caused by the presence of water in the mouth. If the deglutition be caused by a mechanical stimulus with very little or with no liquid thrown into the œsophagus, no peristalsis takes place. If the water thrown into the œsophagus can escape through a longitudinal or transverse cut or finds an obstacle in a ligation, the peristalsis following the swallowing of water progresses just to the cut or the ligature, and no further. This degree of anæsthesia apparently affects the centre of deglutition in such a manner as to stop the progress of the primary afferent impulse within the centre, or to prevent the transmission of its efferent impulses to the œsophagus. The appearance and the progress of the peris-

talsis can now be maintained by direct, short-circuit, reflex contractions caused by the swallowed mass while travelling along its route to the œsophagus.

With a moderate degree of anæsthesia the centre of deglutition is not materially constrained in its function, and the successive contractions of the different parts of the œsophagus are attended to by the primary afferent impulse which started the entire act of deglutition; therefore even an "empty" deglutition is followed by a peristaltic wave, and therefore the peristalsis continues its course even into the part below the division of the œsophagus.

Wild has employed morphine and Mosso ether — not that there is necessarily such a fundamental difference between these two drugs in their anæsthetizing power; the important difference in our case is, as I believe, merely an accidental one. Morphine leaves the body quite slowly, while the animal gets out of the ether-anæsthesia pretty quickly, especially when it can breathe without the obstacle which the glottis and the obstructing parts above it offer to the expiration, *i. e.*, when it breathes through a tracheal cannula. An animal can be deeply anæsthetized through a tracheal cannula within a few minutes and can be out of the anæsthesia in an equally short time. Mosso in nearly all his experiments had the trachea divided, and in some experiments he expressly points out that the animals were almost entirely out of their anæsthesia. Wild on the other hand expressly states that his aim was to have the animal under good anæsthesia, since animals are then, he affirms, reliable reflex-preparations. Wild, in short, observed in his experiments conditions which prevail during deep anæsthesia, and thus discovered the reflex action originating within the œsophagus itself, but failed to see the behavior of the centre of deglutition in normal or nearly normal conditions. Mosso, on the other hand, working under nearly normal conditions, discovered the central nature of the normal progress of the peristalsis, but did not become aware of the existence also of direct reflex mechanisms in the œsophagus, which are perhaps of some assistance even in normal conditions, and surely are of importance as reserve forces when the centre of deglutition is for some reason constrained from exercising its superior function.

I have to add one remark. There might seem to exist some discrepancy regarding a certain point between my statements and those of Wild. I have often seen quoted Wild's assertion that a longitudinal incision does not prevent the progress of the peristalsis, while in

my experiments I have often observed that where a transverse cut prevents the progress of the peristalsis there also a longitudinal incision will prevent it. Wild, however, has made the longitudinal incisions only through the muscular sheaths, taking special care not to injure the mucous membrane, while the incisions I am speaking of were made through the mucous membrane also, thus purposely facilitating the thorough escape of the swallowed masses.

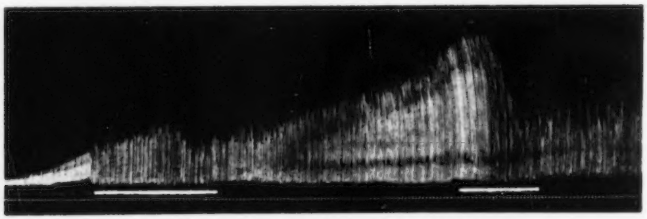

Fig. 2. Supra-renal Extract. (From right to left.)

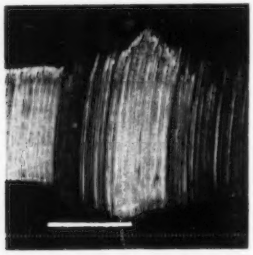

Fig. 3. Kidney. (From left to right.)

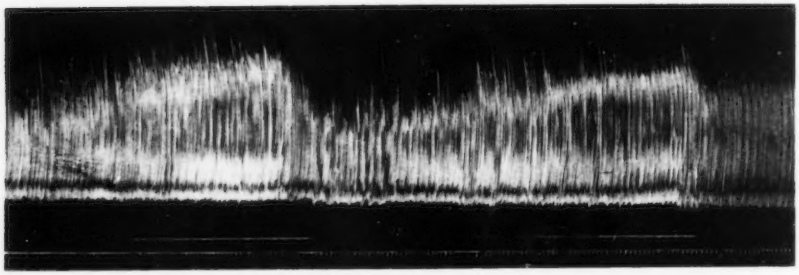

Fig. 4. Thyroid. (From right to left.)

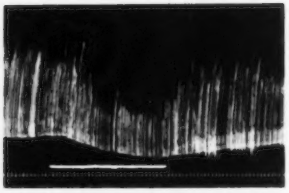

Fig. 5. Thyroid. (From left to right.)

Fig. 6. Anthrax Culture. (From left to right.)

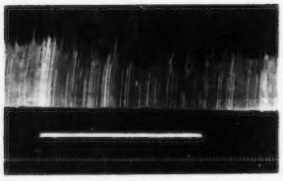

Fig. 7. Filtrate of S. Pyog. Aureus. (From left to right.)

Curves drawn by the "apex" of the dog's heart; one-half the original size. Time in five-second intervals. The white line gives the duration of perfusion.

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THE ACTION OF ANIMAL EXTRACTS, BACTERIAL CULTURES, AND CULTURE FILTRATES ON THE MAMMALIAN HEART MUSCLE.

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CONTENTS.

THE present research on the isolated apex of the dog's heart was undertaken in order to determine the immediate action of glandular extracts and the cultures and filtrates of bacteria on the heart muscle itself.

The influence of nerve cells has been eliminated by using that portion of the heart from which they are absent. The freedom of the apical half of the ventricles from ganglion cells has been recently demonstrated once more by the careful research of Schwartz,[1] in which serial sections of the ventricles of the heart were made in three different planes and new methods of staining employed; nerve cells were found in the ventricles only in the basal third. Any changes produced in the apex preparation by the action of the different substances perfused through it must then of necessity be due to changes in the cardiac muscle itself. There is at present no reason to suppose that these agents act on the heart muscle by stimulating its nerve fibres.

METHOD.

The action of the various animal extracts, filtrates, and bacterial cultures upon the heart muscle free of nerve cells was studied by recording the contractions of the apical portion of the ventricle of the dog's heart kept beating by feeding it with blood according to

[1] SCHWARTZ: Archiv für mikroskopische Anatomie, 1898, liii, p. 63.

Porter's method[1] before, during, and after the perfusion of the apex with the substance to be tested mixed with defibrinated blood.

The preparation of the animal. — The dog received 0.03 to 0.06 gram morphine sulphate hypodermically and fifteen minutes later was anæsthetized with ether. Blood was drawn from the left carotid artery until dyspnœa appeared, when the bleeding was stopped, and a quantity of normally warm 0.8 per cent sodium chloride solution equal to the blood withdrawn was allowed to flow into the right jugular vein. After the saline solution had circulated a few minutes the animal was bled again until dyspnœic. The thorax was hastily opened and the still beating heart rapidly removed and placed in a beaker of warmed normal saline solution, in which it usually continued to contract for some minutes. The blood from the first bleeding was thoroughly defibrinated, filtered twice through glass wool, and divided into portions, one of which was mixed with the product of the second bleeding, while to the other was added an equal quantity of a sodium chloride solution (0.8 per cent) containing the desired proportion of the substance to be tested.

In more than half the experiments, however, the dogs were so large that it was unnecessary to perfuse them in the manner described; it sufficed to bleed them once and to dilute the blood thus gained with an equal quantity of saline solution. A small portion of the undiluted blood was reserved for mixture with the extract of the substance to be tested, which again was always added in such a way that the percentage of blood should be the same in the "normal perfusion fluid" and in the perfusion fluid containing the extract.

A very small glass cannula was now tied into one of the small branches of the coronary artery going to the apex, usually the descendens, and the portion of the ventricle supplied by the branch was cut out and placed in a warm chamber. The cannula was joined to a perfusion tube, and the lower end of the strip of ventricle connected with a writing lever. In some large animals in which the distribution of the arteries was especially favorable as many as three apical preparations could be made from the same heart.

The perfusion apparatus. — The apparatus for carrying on perfusion of the apex, shown in Fig. 1, consisted of a water bottle, a pressure bottle, two blood reservoirs, — one for normal blood and one for blood containing the agent to be tested, — and a chamber in which

[1] PORTER: Journal of experimental medicine, 1897, ii, p. 391.

the apex could be maintained at a constant temperature. The blood reservoirs and the chamber for the apex, with the tubes connecting them, were surrounded with water in a galvanized iron tank. In the upper right hand corner of Fig. 1 is seen the water bottle, supplied with water from a tap, and slung from the ceiling so as to be raised or lowered at will. In the tubulure was fixed a brass tube connected by thick-walled rubber tubing with the pressure bottle. The brass tube was provided with a vertical limb 15 cm. high, open to the

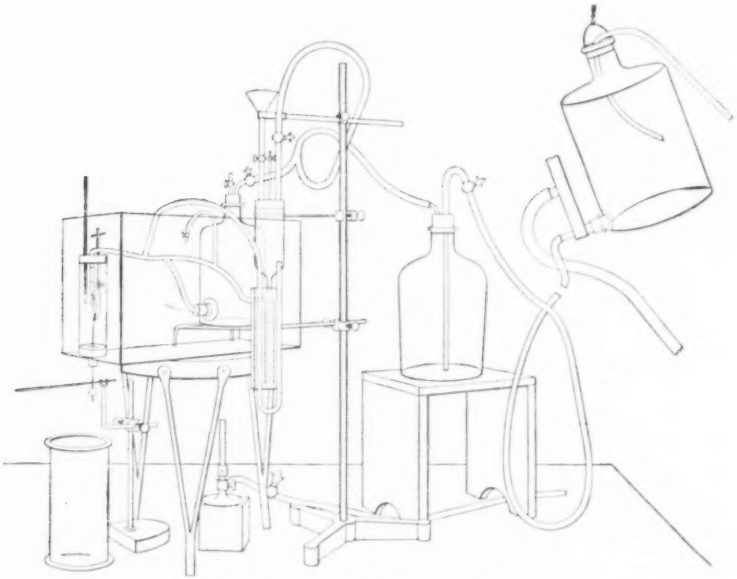

FIGURE 1. Perfusion Apparatus.

air to prevent siphonage. Five centimetres from the top of the vertical limb was a side branch through which the excess of water escaped into a sink. The water in the bottle was thus maintained at a constant level. The pressure bottle is seen on a stand a little to the right of the centre of the figure. Through the cork passed two tubes; one conveyed water from the water bottle already described, the other conveyed the compressed air to the blood reservoirs. The larger blood reservoir contained the normal perfusion fluid, while the smaller held the substance to be tested mixed with defibrinated blood in the desired proportion. Through the stopper of this small reser-

voir ran a glass funnel, which allowed the bottle to be filled without the removal of the stopper. The perfusion tubes given off by the reservoirs united to form the main perfusion tube. The tube from the small reservoir, before it joined with that of the normal reservoir, gave off a side branch, the end of which was closed by a small stopcock; this allowed the small bottle to be washed out without interrupting the normal perfusion, so that each toxic substance could be thoroughly removed before another was added. About five centimetres beyond the junction of these perfusion tubes the main tube gave off a branch to a mercury manometer by which the pressure of the perfusion fluid was recorded. The main tube then ran directly to the chamber for the apex — seen at the left of the tank with a piece of muscle ready for perfusion — where it was connected with the cannula in the branch of the coronary artery. In the stopper of the warm chamber were fixed a thermometer and an adjustable clamp to hold the strip of apex in position. The lower end of the apex chamber had a small opening for the escape of the blood which had passed through the vessels of the preparation. This opening served also for the wire connecting the apex with the recording lever, which was of the third class and magnified seven times. When the apparatus was in use the heart chamber, blood reservoirs, and perfusion tubes were submerged in water of a temperature of 37.5° C. No reference has been made to the stopcocks seen in Fig. 1, as their uses can readily be understood. No stopcocks were used on the perfusion tubes, the circulation through them being controlled with long-handled artery forceps. The recording lever marked on the smoked surface of a Baltzar drum revolving very slowly. An electrical time marker was used to indicate every five seconds.

The preparation of the glandular extracts. — The glandular extracts were in all cases prepared from perfectly fresh material dissected from newly killed sheep at the slaughter house.

Both glycerine and saline extracts were employed. The former is invariably the more powerful.

In preparing the glycerine extract the fresh glands were stripped of fat and other extrinsic material, weighed while in a moist state, cut into small pieces, placed in a glass vessel, and covered with chemically pure glycerine in the proportion of one gram of gland substance to one cubic centimetre of glycerine. After allowing the gland to remain in this for twenty-four hours the glycerine extract was strained through cheesecloth and added to boiled 0.8 per cent sodium

chloride solution in the desired proportion. The mixture was then filtered, and on the addition of an equal quantity of pure defibrinated blood was ready for perfusion. The perfusion fluid always consisted of fifty per cent blood and fifty per cent normal saline solution, but the quantity of glycerine extract dissolved in the saline solution varied. The usual proportion was glycerine extract 5 c.c., saline solution 45 c.c., blood 50 c.c.

The saline extracts were prepared by rubbing up the desired quantity of the gland substance in a mortar with 0.8 per cent sodium chloride solution and a small quantity of clean white sand. The mixture was then gently warmed, filtered, and added to the blood in the proportion required. No extracts were prepared from dried glands.

The preparation of cultures and filtrates. — The cultures of bacteria used in these experiments were grown on dog's serum. An attempt to use bouillon cultures had to be abandoned because the presence of small traces of the calcium and potassium salts in the fluid so altered the contractions as to make an accurate judgment of the effects of the bacteria or filtrates impossible.

The serum for the cultures was secured by bleeding the animal into a sterilized vessel, which was set aside, protected from infection by having its mouth covered with several sheets of sterilized paper, until the serum had separated. The serum was then drawn off and put in several sterilized flasks, which were placed in an incubator and kept at a constant temperature (38° C.) for seven days. If at the end of that time the serum remained sterile the bacteria were planted in it and the cultures allowed to grow in an incubator for ten days. Then the contents of each flask was divided into two portions; one was left as it was, the other was passed through a Chamberland filter to remove the bacteria. This was done to determine if any difference in the character or strength of action existed between a culture containing the bacteria and its filtrate. Both the culture and the filtrate were added respectively to the normal saline solution, as already described in the case of the glandular extracts.

Control experiments were done with all the glycerine, saline solutions, and serum employed in the research. The first two substances gave no results; the serum usually increased very slightly the force of the contractions.

The perfusion fluid used in these experiments was the animal's own defibrinated blood mixed with an equal quantity of sodium chloride

solution (0.8 per cent). The apex contracts equally well when perfused with its own undiluted blood, but the addition of the saline solution secures certain advantages; it gives a larger quantity of fluid to work with, an important matter when the experiment occupies six or seven hours; it also allows the experimental substance to be added to the perfusion fluid at the expense of the inert saline solution, and so does not alter the percentage of defibrinated blood. In control experiments it was found that the saline solution had no action on the contracting apex of itself, and when mixed with the defibrinated blood in varying proportions its only action was that of a diluent. That is to say, pure defibrinated blood gave strong apical contractions and the contractions were not affected in any appreciable degree when saline solution was added to the extent of fifty per cent; but beyond that the contractions of the apex became correspondingly weaker as the percentage of saline was increased, and on the perfusion of pure saline no response or at best a very weak and short-lived activity was developed.

In all experiments the blood was examined under the microscope to see if the experimental substance had produced any change in the corpuscles. In only one case was any noteworthy change detected; in this instance a culture of glanders had been added to the blood, and the corpuscles showed a tendency to stick together and so form small clumps. The extracts of glands did not alter the usual characters of the corpuscles; in all cases they looked entirely normal.

In these experiments the apex was kept at a constant temperature, namely, that of the body, and the pressure of the perfusion fluid was also constant — 80 mm. Hg.

With regard to the behavior of the apex preparation under normal perfusion (defibrinated blood diluted with an equal part of normal saline solution) it was found, in the greater number of cases, that the contractions were fairly regular in rate; the force varied more than the frequency, but not to such an extent as to impair the accuracy of the conclusions drawn in this paper. In one experiment only was the apex perfused with an animal extract while the contractions were irregular, and this was done merely to show the regulating influence of the extract (thyroid) on the rhythm.

An excess of the substance perfused sometimes caused the apex to fibrillate, but by entirely shutting off the perfusion and so asphyxiating the apex for a short time it was found that when the normal perfusion was resumed the apex as a rule returned to its regular con-

tractions. This method of recovering the apex from fibrillary contractions was first pointed out by Langendorff.[1]

During the course of this inquiry thirty dogs were used; always two and sometimes three preparations were made from each heart. The extract of each of the glands investigated was used upon a fresh apex preparation, — one not previously employed for any purpose, — but more than one perfusion was made with each extract. Sometimes perfusion was done also on an apex which had been perfused before with some other extract, but this only when the apex had not been visibly affected or had recovered from the action of the foregoing substance. The table on page 280 gives the number of perfusions done with each substance.

The Action of the Extracts of Various Organs on the Heart.

Suprarenal bodies. — The action of suprarenal extracts on the circulation was first fully demonstrated by Oliver and Schäfer,[2] who pointed out that its influence on the heart and blood pressure is largely a direct one, and that the medulla of the gland contains the active substance which on injection causes a rise in blood pressure and augmentation of the heart-beats. Among the more important subsequent investigations may be cited Hedbom's[3] research with the whole isolated cat's heart, in which the extract was perfused through the coronary vessels; here it was found that the suprarenals exerted a strong tonic influence on the contracting heart, and that the effect lasted for a long time. Cybulski,[4] Szymonowicz,[5] and Gottlieb[6] state that the extract of these bodies affects the heart through the nervous system. The last named observer pointed out that the substance is also a powerful cardiac stimulant; he revived the heart of a rabbit with suprarenal extract from the arrest brought on by the intravenous injection of chloral hydrate.[7] Vincent[8] has demonstrated on dogs that the suprarenals have no power when fed to animals even in enor-

[1] Langendorff: Archiv f. d. ges. Physiol., 1895, lxi. p. 319.
[2] Oliver and Schäfer: Journal of physiology, 1895, xviii. p. 230.
[3] Hedbom: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.
[4] Cybulski: Akademie d. Wissensch., Cracovie, 1895, 4 Mars.
[5] Szymonowicz: Archiv f. d. ges. Physiol., 1896, lxiv, p. 97.
[6] Gottlieb: Archiv f. exper. Pathol. und Pharmakol., 1896, xxxviii, p. 99.
[7] Gottlieb: *Ibid.*, p. 106.
[8] Vincent: Journal of physiology, 1898, xxii, pp. iii, 270, lvii.

Substance.	Total Number of Perfusions.	On fresh apex preparation.		On Apex already Used.	
		First Time.	Repetitions.	Times.	Previous perfusion.
Extract.					
Suprarenal	11	7	3	1	Thyroid extract.
Pituitary body . . .	6	2	2	2	Testicular extract, infundibular extract.
Infundibular body .	8	2	2	4	Testicular extract (4).
Testicle	11	5	2	4	Pituitary, infundibular, and thyroid (2) extracts.
Liver	9	6	1	2	Pancreas, spleen.
Pancreas	8	6	1	1	Submaxillary gland.
Submaxillary gland .	9	6	2	1	Spleen.
Spleen	8	6	1	1	Liver.
Kidney	8	4	4	0	
Thyroid	24	10	10	4	Testicular extract (2), suprarenal extract (2).
Culture or its Filtrate.					
Staphy. pyo. aureus .	6	3	3	0	
Culture filtrate . .	4	2	2	0	
Typhoid	5	1	1	3	Glanders, prodigiosus, aureus.
Culture filtrate . .	4	1	1	2	Capsulatus, aureus.
Prodigiosus	4	1	1	2	Aureus (2).
Culture filtrate . .	1	0	0	1	Prodigiosus.
Glanders	2	1	0	1	Typhoid.
Culture filtrate . .	3	1	0	2	Glanders, capsulatus.
Diphtheria	2	1	1	0	
Diphtheria toxine .	5	3	2	0	
Cholera spirillum . .	3	1	1	1	Aureus culture filtrate.
Culture filtrate . .	1	0	0	1	Aureus.
Capsulatus	5	1	2	2	Anthrax, tetanus toxine.
Culture filtrate . .	2	1	1	0	
Anthrax	5	2	1	2	Tetanus toxine, pyocyaneus.
Culture filtrate . .	3	2	0	1	Diphtheria toxine.
Pyocyaneus	2	0	0	2	Cholera spirillum, aureus.
Culture filtrate . .	1	0	0	1	Aureus culture filtrate.
Megatherium . . .	2	0	0	2	Coli communis (2).
Culture filtrate . .	1	0	0	1	Diphtheria toxine.
Coli communis . .	2	0	0	2	Prodigiosus, anthrax.
Culture filtrate . .	1	0	0	1	Diphtheria toxine.
Ramosus	2	0	0	2	Anthrax culture filtrate.
Culture filtrate . .	0	0	0	0	
Tetanus toxine . .	5	2	1	3	Pyocyaneus culture filtrate, anthrax culture filtrate.

mous quantities, but the giving of a minute dose intravenously immediately causes marked changes in the cardiac action and in the blood pressure.

The nature of the active principle of these bodies has been much discussed. Muhlmann[1] attributed the action to the presence of pyrocatechin. Langlois[2] contradicts this, and in an interesting paper in collaboration with Charrin[3] shows that the suprarenal extract is capable of diminishing the poisonous effects of nicotine. Abelous[4] points out that it is also antagonistic to the action of atropine.[5]

Of all the glandular extracts used in my experiments that of the suprarenal capsules is by far the most powerful; in the seven experiments done with this substance not once did it fail to produce a marked augmentation of the contractions of the apex.

In the experiment illustrated by Fig. 2, Plate I, the dog was anæsthetized with morphia and ether at 9 A. M. Fifteen minutes later he was bled from the left carotid artery. At 9.45 the heart was removed and at 9.52 normal perfusion was commenced; the apex showed strong contractions as soon as the perfusion began. At 9.59 the suprarenal mixture was allowed to pass through the apex. This liquid consisted of 3 c.c. of a glycerine extract made from the mixed gland, *i. e.* cortex and medulla, 47 c.c. sodium chloride solution (0.8 per cent), and 50 c.c. defibrinated blood. At 10.01 this toxic fluid was shut off and normal perfusion resumed; after six minutes the suprarenal extract was again turned on for three minutes, but, as the apex showed great excitement, resort was had to the normal perfusion fluid, — too late, however, to prevent the apex from fibrillating, as can be seen at the end of the tracing.

In all experiments the apex answered almost immediately to the

[1] Mühlmann: Deutsche medicinische Wochenschrift, 1896, xxvi, pp. 409-411.

[2] Langlois: Archives de physiologie, 1897, xxix, p. 152.

[3] Charrin and Langlois: Bulletin de la société de biologie, 1893, p. 842, 1894, p. 99, 1896, p. 131.

[4] Abelous: C. r. de la société de biologie, 1896, p. 458.

[5] Since writing the above I have had the pleasure of hearing Dr. J. J. Abel's paper, read before the American Physiological Society, December 28, 1898, on the active principle of the medulla of the suprarenal glands. This epinephrin, as Dr. Abel calls it, has the formula $C_{17}H_{15}NO_4$ and is to be classed with the alkaloids. Its sulphate is very active; a dose of 0.00013 gram, equal to 0.00009 gram of the free base, produced a rise of 14 mm. Hg in the blood pressure in a small dog. Dr. Abel's paper will be found in the Bulletin of the Johns Hopkins Hospital, and in full in the Zeitschrift fur physiologische Chemie, 1899.

presence of the extract, the contractions being often more than double the height of the normal ones (see Fig. 2) while the rhythm was slightly quickened. No alteration in the tonus was noticed unless the perfusion was carried on for some time, when a gradual rise took place until the apex ran into fibrillary contractions. Provided the first dose was of short duration, about two minutes, and not too strong, the effect gradually wore off, and, as is shown in Fig. 2, the heart did not respond to a second dose in so marked a manner or so readily as to the first. A strong dose of the extract, about ten per cent (saline extract), would as a rule cause the apex to fibrillate after giving four or five enormous contractions.

Hypophysis cerebri. — Oliver and Schäfer[1] called attention to the action of the extract of the hypophysis when injected into the circulation. They obtained marked results, the blood pressure rising considerably, the heart's action being slowed, and the contractions augmented; a large dose was required to produce these effects, however. Mairet and Bosc[2] failed to obtain any pronounced effects after injections of the hypophysis. Hedbom[3] found in his research a tonic influence on the heart and a decrease in the frequency of the contractions. Cyon[4] found a slowing action on the heart in consequence both of stimulation of the hypophysis *in situ* and of injections of its extract. He believes that the active substance, though in the main similar to that of the thyroid, differs in that it contains phosphorus.

Under the heading "Hypophysis cerebri" I have thus far included two different structures, namely, the pituitary body proper and the infundibulum. The necessity of considering these structures separately has been pointed out by Howell,[5] who calls attention to the fact that all experiments with the hypophysis, previous to his own, have evidently been done with the infundibular and pituitary bodies combined.

In separate experiments with extracts of these two structures Howell found a great difference in their action. Little or no result was obtained with the pituitary body, while an injection prepared from the

[1] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 277: also Schäfer: British medical journal, 1895, Aug. 10: and Text-Book of physiology, edited by Schäfer, 1898, i, p. 946.

[2] Mairet and Bosc: Archives de physiologie, 1896, viii, p. 600.

[3] Hedbom: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.

[4] Cyon: Archiv f. d. ges. Physiol., 1898, lxx, p. 126.

[5] Howell: Journal of experimental medicine, 1898, iii, p. 245.

infundibular body gave constant and well marked effects, slowing the rate of contraction of the heart and raising the blood pressure. Moreover, it has been shown by Haller[1] and Berkley[2] that the histological structure of the infundibulum and pituitary body differs widely and that their embryological origin is dissimilar also. Berkley found in the infundibular body not only nerve structures but also closed vesicles lined with gland-like epithelial cells and containing a material like colloid. This substance has been shown by Howell to possess physiological activity.

These experiments confirm Howell's observations on the difference of action between these two substances. The perfusion of a glycerine extract (10 per cent) of the pituitary body alone caused an almost immediate change in the contractions of the apex; the frequency was considerably lessened but the amplitude of the contractions was increased; no alteration in tonus was noticed, but a small alteration may have been masked by the slight irregularity in the action of the apical preparation. No after effects were observed, the ventricular strip returning to its normal action on resuming normal perfusion. The change in the amplitude and frequency of the contraction began to disappear, as a rule, before the perfusion of the pituitary extract had been shut off; in short, the result was only momentary.

The results with an extract prepared from the infundibular body were very different. On perfusion of a two per cent solution the beats were less frequent and decreased in amplitude, and the tonus fell a little and then sometimes rose considerably. No after effects were observed, and a second dose did not appear to be as potent as the first.

The infundibulum seemed to be the more powerful of the two bodies, for the heart was affected by a much smaller dose (two per cent) than in the case of pituitary portion, which usually required to be made much stronger (ten per cent) before any change could be detected in the character of the apical contractions.

Orchitic extract. — In 1889 Brown-Séquard[3] published the results of a series of experiments with orchitic extract upon himself. He declared that the power of his voluntary muscles was considerably increased by the injections made. The increase of power he considered to be not the result of a simple stimulus, as the effect con-

[1] HALLER: Morphologisches Jahrbuch, 1896, xxv, p. 31.

[2] BERKLEY: The Johns Hopkins hospital reports, 1895, iv, p. 285.

[3] BROWN-SÉQUARD: Archives de physiologie, 1889, xxi, p. 651.

tinued too long for this, but rather to be of a dynamogenic nature. That this substance also exercises, in some states, a beneficial influence on the blood has been shown by Hénocque,[1] who found that in phthisical patients a permanent increase in the quantity of hæmoglobin takes place. Poehl[2] prepared from the testicles a substance he calls "spermine," in which he finds high oxidizing powers. De Tarchanoff[3] found that the administration of "spermine" rendered the heart resistent to the effects of chloroform. Brown-Séquard[4] pointed out however that neither this substance nor the Charcot-Naumann crystals are the active principle of orchitic extract. On the isolated cat's heart Hedbom[5] noted that orchitic extract produces increased frequency and force of the cardiac contractions; the effect lasts for some time.

In my own experience the result of perfusing orchitic extract has varied according to the dose. A small dose, four per cent, of glycerine extract, simply caused an increase in the force of the apical contraction with no visible change in tonus or rhythm. A larger dose, on the other hand, caused a marked fall in tonus, and a few moments later a quickening in the rhythm; the contractions were also slightly increased in force.

The changes produced by a small dose made their appearance almost immediately and disappeared on resuming the normal perfusion. With a stronger dose, ten per cent, the fall in tonus occurred with the beginning of orchitic perfusion and passed off as soon as normal blood was perfused, while the increase in frequency and force lasted for some time after.

Liver. — The liver apparently contains nothing soluble in glycerine or saline solution that affects the musculature of the heart. In six experiments the perfusion of saline solutions up to thirty per cent strength produced no change in force or frequency and caused but a slight fall in tonus, which passed away as soon as the apex was supplied with the normal perfusion fluid.

Pancreas. — That the pancreas secretes some material necessary to the normal functions of the body is of course well known. Notwithstanding this the perfusion of the apex with blood containing less

[1] Hénocque: Archives de physiologie, 1892, xxiv, p. 45.
[2] Poehl: Berliner klinische Wochenschrift, 1893, Nr. 36.
[3] De Tarchanoff: Archives ital. de biologie, 1895, xxii, p. xxxix.
[4] Brown-Séquard: Archives de physiologie, 1891, xxiii, p. 400.
[5] Hedbom: Skandinavisches Archiv für Physiologie, 1898, viii, p. 147.

than twenty per cent of the saline extract gave little or no result in six experiments. But when the perfusion fluid contained twenty per cent or more of the extract the contractions were lessened in frequency and a fall in tonus was produced. The apex recovered shortly after resuming the normal perfusion.

Submaxillary gland. — An extract of the submaxillary gland of the same strength and character as the pancreas extract was perfused with a very similar result, the only difference being that the fall in tonus was not quite so well marked. Hedbom[1] found the action of this gland to be tonic in its nature on the whole isolated cat's heart. In six experiments I failed to find anything but a depressing effect in these two substances.

Spleen. — That the spleen has some sort of influence on the work of the pancreas was pointed out by Schiff,[2] in 1862, and more recently Gachet and Pachon[3] state that the internal secretion of the spleen modifies the amylolytic secretion of the pancreas. Oliver and Schäfer[4] find that an extract of the substance of the spleen injected into an animal produces a fall in blood pressure soon followed by a rise and then a slow gradual return to the normal. Hedbom[5] observed that the splenic extract increases the tonus of the heart muscle and tends to regulate the rhythm.

In the present investigation a splenic glycerine extract of ten per cent strength produced a slight fall in tonus and slightly increased the frequency and strength of the contractions. It also appeared to make the rhythm more regular. On returning to normal perfusion the contractions became still more forcible, but this effect gradually passed off and the contractions resumed their normal appearance. The above results were, however, far from constant; in about forty per cent of the experiments with this extract little or no change in the character of the contraction was noticed.

The results obtained with the four organs thus far considered, liver, pancreas, submaxillary gland, and spleen, are hardly as satisfactory as could be wished. To produce an effect the extracts had to be given in very large doses, and even then the result was not always well marked.

[1] Hedbom: *loc. cit.*

[2] Schiff: quoted by Gley: Archives de physiologie, 1892, xxiv, p. 391.

[3] Gachet and Pachon: Archives de physiologie, 1898, xxx, p. 364.

[4] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 278.

[5] Hedbom: *loc. cit.*

Kidney. — That the kidney has an internal secretion was pointed out by Brown-Séquard,[1] and the same observer in collaboration with d'Arsonval[2] showed that injections of kidney extract, prepared in a similar manner to orchitic extract, is of benefit when the internal secretion of the kidney is deficient, either from extirpation or extensive disease of the organ. Meyer,[3] from observations made mainly on the respiratory system, confirmed this statement. These observers also showed that although the urine may be completely suppressed for some time, fatal results do not by any means immediately ensue, in fact the suppression may exist for weeks; on the other hand death rapidly follows the extirpation of both kidneys; finally, in these cases of double extirpation animals will live for a much longer time than otherwise if given injections of kidney extract. Bradford's[4] experiments also demonstrate the importance of the kidney in vital processes other than the mere excretion of urine. In short, it appears from these investigators that the kidney is not merely a simple remover of waste products, but that it also produces a substance stimulating to anabolic processes and obstructive to tissue disintegration.

Tigerstedt and Bergman[5] more recently have shown that the injection of kidney extract into the circulation causes a slight fall in the blood pressure followed by a considerable rise. They did not find that the kidney extracts much affected the heart isolated by Langendorff's method.

In these experiments, done on the hearts of two animals and with two apical preparations from each heart, there was found a decided alteration in the character of the contraction. Perfusion of solutions from two to eight per cent strength caused a drop in tonus and a slight slowing of the rate of contraction (Fig. 3). The slowing seen in Fig. 3 is more prominent in this tracing than in any other obtained, although it was present in all. As may be observed, it was soon succeeded by much more forcible contractions of about the normal frequency. On perfusion with normal blood the apex quickly returned to its usual beat.

[1] Brown-Séquard: Archives de physiologie, 1893, xxv, p. 778.

[2] Brown-Séquard and d'Arsonval: Comptes rendus de l'academie des sciences, 1892, cxiv, p. 400.

[3] Meyer: Archives de physiologie, 1893, xxv, 760; and 1894, xxvi, p. 179.

[4] Bradford: Journal of physiology, 1891, xii, p. xviii.

[5] Tigerstedt and Bergman: Skandinavisches Archiv für Physiologie, 1898, viii, p. 223.

Perfusion of urea dissolved in the normal saline solution was also tried, and in amounts up to 2.5 per cent was found to act invariably as an excitant: the contractions were augmented and accelerated but irregular in character; a slight rise in tonus took place.

Thyroid.[1] — On injecting an extract of the thyroid gland Schäfer[2] obtained a fall in blood pressure but no change in the heart-beat. Cunningham[3] on the other hand observed not only a fall in blood pressure but also an increased force of contraction. Hutchinson[4] found that the extract had no effect on the heart, but obtained a fall in blood pressure. The colloid material of the gland gave him negative results, — only the salts were active. Artificial compounds of iodine and albumin are without effect according to this observer. Vomossy and Vas[5] and Kobert[6] declare that thyro-iodine has no effect on the heart, and Schuster[7] finds that even large doses cause no alteration in the pulse in man. Mossé[8] found by means of the ergograph that the work done by the voluntary muscles was increased by the administration of thyro-iodine; the onset of fatigue appeared to be postponed. Cyon[9] asserts that the intravenous injection of thyro-iodine augments the excitability of the vagi, while the injection of iodine exerts an opposite effect: *i. e.*, increases the excitability of the sympathetic, quickens the heart, and raises the blood pressure. He therefore concludes that thyro-iodine is a direct antagonist of iodine.

In my experiments, the perfusion of a fluid containing three per cent of a glycerine extract of the thyroid gland considerably augmented the force and slightly quickened the rate of contraction, but the effect gradually wore off (Fig. 4). It was also noticed that the extract appeared to exert a regulating influence on the rhythm. Small doses of saline extract gave exactly similar results. Large doses of saline extract, 15 to 20 per cent, had in most respects a contrary

[1] Reported in abstract to the Boston Society of Medical Sciences and published in their journal, 1898, iii, p. 58.

[2] Schäfer: British medical journal, 1895, p. 343.

[3] Cunningham: Journal of experimental medicine, 1898, iii, p. 148.

[4] Hutchinson: Journal of physiology, 1898, xxiii, p. 178.

[5] Vomossy and Vas: Münchener medicinische Wochenschrift, 1897, Nr. 25.

[6] Kobert: Verhandlungen des 14ten Congresses fur innere Medicin, 1896, p. 153.

[7] Schuster: Wiener medicinische Wochenschrift, 1896, p. 379.

[8] Mossé: Archives de physiologie, 1898, xxx, p. 742.

[9] Cyon: Archiv f. d. ges. Physiol., 1898, lxx, p. 126.

effect; there was a marked fall in tonus and the contractions became considerably less forcible. But the rhythm, as in the case of the weaker extracts, appeared to be more regular during the perfusion than either before or after (Fig. 5). Continued perfusion of the stronger extracts would eventually paralyze the apex.

Thyro-iodine, prepared according to Baumann's method[1] and dissolved by the aid of sodium bicarbonate, when mixed in small or large amounts with the perfusion fluid produced effects on the apex exactly similar to those caused by glycerine or saline extracts.

In order to test the regulating influence of the thyroid extract on the rhythm, the apex was perfused while the contractions were extremely irregular. The result was an immediate slight fall in tonus. The beat then became a little more forcible and more regular, and, as the thyroid perfusion was stopped and the normal perfusion resumed, the ventricular strip beat in excellent rhythm and slightly quicker than during the thyroid perfusion.

A solution of iodine (0.05 per cent) dissolved in sodium chloride solution (0.8 per cent) was also tried, but the results were entirely different from those obtained with either thyro-iodine or extracts of the gland. At first the beats were increased in size, but they gradually fell to their normal before the cessation of the perfusion; a slight rise in tonus took place; the apex became very irregular in its contractions, and the irregularity continued a considerable time after resuming normal perfusion.

The Action of the Cultures and Culture Filtrates of Bacteria.

Several observers have obtained from toxines direct results on the heart, but most of these were due to degenerative changes and some time necessarily elapsed before the effects became marked. Charrin[2] produced by the injections of toxines such degenerative changes in the heart that heart failure took place. This same observer, with Bardier,[3] in a series of experiments made upon frogs, found that six

[1] Baumann and Roos: Zeitschr. f. physiol. Chemie, 1896, xxi, p. 481. For the preparation of the thyro-iodine used in these experiments I am indebted to the kindness of Dr. Alfred W. Balch, of the Pharmacological Laboratory, Harvard Medical School. The thyro-iodine was prepared from perfectly fresh glands and used at once.

[2] Charrin: C. r. de la société de biologie, 1896, p. 867.

[3] Charrin and Bardier: Archives de physiologie, 1897, xxix, p. 554.

hours after an intraperitoneal injection of 1 c.c. of diphtheria toxine, the heart was slowed; alteration in the temperature did not modify this action. The effect, they remark, may be due to stimulation of the vasomotor centre. Enriquez and Hallion [1] assert that the toxine of diphtheria paralyzes the inhibitory nerves of the heart while the accelerator nerves preserve their action. Roger [2] found, in frogs, that the bacillus diphtheriae lessened both the force and frequency of contraction. Large doses were required to produce the result, which even at the best was not well marked. Another organism, which he speaks of as bacillus septicus putidus, possessed this depressant action in a powerful degree. Sharp [3] removed the frog's heart and passed through it the products of a culture tube of the Klebs-Loeffler bacillus mixed with his perfusion fluid; a prolonged diastole and subsequent heart failure was the result.

The following bacteria and their filtrates were used in the present experiments: Staphylococci pyogenes aureus, anthrax, coli communis, pyocyaneus, typhoid, megatherium, cholera spirillum, capsulatus, diphtheria, ramosus, prodigiosus, and glanders.[4] All of the above bacteria, pathogenic and non-pathogenic, when perfused unfiltered, exercised a depressant action on the apical preparation, almost immediately slowing the rate and weakening the force of the contractions. The result seemed to vary according to the size of the bacterium; cultures of large bacteria giving the most pronounced effect, while smaller ones did not produce so great a change. Fig. 6 shows the abrupt diminution in force and frequency of the contractions produced by the perfusion of a culture of anthrax. The quick recovery of the apex is remarkable. If the perfusion of the bacteria is carried on too long, however, either with pathogenic or non-pathogenic organisms, the apex very often fails to recover its normal, regular contractions.

The action of the filtrate on the apex was entirely different in several particulars from that of the bacteria; first, no results were obtained, in doses up to thirty per cent, from filtrates of non-pathogenic bacteria; secondly, the action obtained from the perfusion of

[1] Enriquez and Hallion: Archives de physiologie, 1898, xxx, p. 393.

[2] Roger: C. r. de la société de biologie, 1893, p. 175.

[3] Sharp: Journal of anatomy, 1897, xxxi, p. 199.

[4] I am indebted to the kindness of Dr. A. K. Stone of the Bacteriological Laboratory, Harvard Medical School, for the preparation of the cultures, toxines, and filtrates.

pathogenic filtrates came on slowly, was depressant in its character, and passed off slowly when normal perfusion was resumed. It was necessary to give the filtrate in large doses to produce these effects, and when the perfusion was carried on for some time the apex quickly lost its contractile power (Fig. 7).

The conclusion I am forced to draw from the above phenomena is that the action of the bacteria on the contracting apex is mainly mechanical. Combined with this, in the case of pathogenic bacteria, is the depressant influence of their filtrates. The pathogenic filtrates are purely depressant; their action possibly was partly hidden by the stimulating properties of the serum proteids contained in the solution.

Very powerful fresh toxines of tetanus and diphtheria were also employed. The latter, when perfused in doses of 25 per cent, slowed the rate but did not greatly alter the force of beat; the tetanus toxine on the other hand was absolutely without effect, even in solutions containing 40 per cent.[1]

This research was undertaken at the suggestion of Professor W. T. Porter, and I am greatly indebted to his kind criticism of the results obtained.

[1] For these I must thank Professor Theobald Smith, of the Laboratory of Comparative Pathology, Harvard University.

THE FORMATION OF MELANINS OR MELANIN-LIKE PIGMENTS FROM PROTEID SUBSTANCES.

By R. H. CHITTENDEN and ALICE H. ALBRO, Ph. D.

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THE brownish black animal pigments, collectively known as melanins, occurring normally and pathologically in the body, are characterized as a class by a somewhat high content of carbon, a relatively low content of nitrogen, and a variable, though usually high, percentage of sulphur. The presence of the latter element in very appreciable amounts constitutes one of the reasons for the belief that these substances have their origin in some proteid antecedent, while the absence of iron, in most cases, excludes the view that they originate from the blood pigment. In a recent contribution by Schmiedeberg[1] to the chemical composition and nature of melanins, emphasis is laid upon the possible origin of these substances in antialbumid. This view is seemingly based upon the partial resemblance in chemical composition between the latter substance and the melanins; a resemblance which is indeed somewhat striking. As a cleavage product of the proteids, antialbumid is characterized by a comparatively high content of carbon and a low content of nitrogen, and Schmiedeberg concludes that melanin may result from antialbumid by a process of hydrolytic cleavage comparable to the method by which the latter body results from albumin itself. He further points out that the sulphur of the antialbumid may remain with the melanin, thus accounting for the large amount of this element usually found, while ammonia and water are split off according to the following equation: —

$$\underset{\textit{Antialbumid.}}{C_{120}H_{187}N_{27}SO_{37}} = \underset{\textit{Melanin.}}{C_{120}H_{114}N_8SO_{29}} + 19\,NH_3 + 8\,H_2O.$$

This formula for antialbumid is based upon an analysis of antialbumid prepared by Kühne and Chittenden[2] from serum-albumin, while the formula for the melanin is based upon the analysis of a

[1] Schmiedeberg: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 65.

[2] Kühne and Chittenden: Zeitschrift für Biologie, 1883, xix, p. 176.

product (melanoidic acid) prepared by Schmiedeberg[1] by boiling serum-albumin with 25 per cent hydrochloric acid for twelve hours. Schmiedeberg likewise prepared a melanoidic acid from the fibrinoses contained in Witte's "pepton" by heating these proteoses with phosphoric acid for two months. Ascribing to the proteoses present in Witte's "pepton" a formula with 102 atoms of carbon, he formulates the production of the melanin as follows:

$$\underset{\textit{Fibrinose.}}{C_{102}H_{150}N_{30}SO_{31}} + 18\,H_2O = \underset{\textit{Melanin.}}{C_{102}H_{98}N_{12}SO_{32}} + 18\,NH_3 + 17\,H_2O.$$

It is to be observed that these two artificial melanins or melanoidic acids differ from each other by nearly 6 per cent of carbon and 3 per cent of nitrogen, and that sulphur was determined in only one of the products, the amount found being 0.96 per cent. While the data may be considered as perhaps hardly sufficient to justify such definite expressions as the above formulæ, the general trend of the argument is exceedingly interesting and important. Any one who has worked much with the proteids is aware how readily these substances yield dark colored solutions on boiling with dilute mineral acids; a reaction not due to oxidation alone since it takes place readily even in the presence of reducing agents. It is thus evident that melanin-like substances may be formed from proteids by simple hydrolytic cleavage, and consequently the question at once arises how far the nature of the mother substance modifies the character of the resultant pigment. Further, is one proteid better adapted for the artificial production of a melanin than another proteid? How closely do the artificial melanins resemble in composition and general characters the natural pigments of this class? And lastly, how far can reactions of this kind be accepted as indicating the mode of formation of the natural pigments common to the body in health and disease? Some of these questions we have endeavored to answer by the following experimental work.

Formation of Melanin from Antialbumid. — In view of the somewhat noticeable parallelism in composition between antialbumid and the melanins, attention was first directed to the possible formation of a pigment of this class by hydrolysis of antialbumid. For this purpose a pure antialbumid of known composition was necessary, and since the latter body is prone to slight alteration by too vigorous hydrol-

[1] SCHMIEDEBERG: *loc. cit.*, p. 66.

ysis particular attention was paid to the conditions attending its formation. As the mother substance in the preparation of antialbumid, thoroughly washed, coagulated egg-albumin was employed. In the hydrolysis, 2600 grams of the moist coagulum, pressed as dry as possible, were heated with 7 litres of 3.0 per cent sulphuric acid in a large flask, filled quite to the neck to diminish oxidation, at 100° C. (in a large Arnold sterilizer) for 10 hours. The gelatinous mass of impure antialbumid was thrown upon filters, allowed to drain, and after some washing with water, was transferred to a flask and heated with 3.5 per cent sulphuric acid for 10 hours, after which it was again filtered off and washed with water — by decantation and otherwise — until the washings were nearly or quite free from acid reaction. To remove any traces of unaltered proteid possibly present, as well as other impurities, the antialbumid was next warmed at 38° C. for 30 hours with an active solution of pepsin-hydrochloric acid (0.2 per cent HCl), the soluble products removed by filtration, and the residual antialbumid washed with water until the washings gave no reaction with the biuret test, nor with silver nitrate for chlorine. The washing was facilitated, after the bulk of the soluble matter had been removed, by carefully adding to the antialbumid suspended in water sufficient dilute solution of potassium hydroxide to make the mixture quite neutral to test paper, thus aiding the removal of any loosely combined sulphuric acid. Finally it was washed with weak and strong alcohol and lastly with ether. When dry, the finely powdered substance was repeatedly boiled with distilled water to insure the complete removal of any adherent soluble salts.

Analysis of a sample of this product dried at 110° C. until of constant weight gave the following results:

I. 0.3397 gram substance gave 0.2098 gram H_2O = 6.86 per cent H and 0.6654 gram CO_2 = 53.45 per cent C.

II. 0.3439 gram substance gave 0.2114 gram H_2O = 6.83 per cent H and 0.6719 gram CO_2 = 53.29 per cent C.

III. 0.2259 gram substance gave by the Kjeldahl method 0.03054 gram N = 13.52 per cent N.

IV. 0.2502 gram substance gave 0.03429 gram N = 13.70 per cent N.

V. 0.6210 gram substance gave by fusion with NaOH[1] and KNO_3 0.1001 gram $BaSO_4$ = 2.21 per cent S.

VI. 1.0297 grams substance gave 0.0031 gram ash = 0.30 per cent.

[1] Pure NaOH prepared from metallic sodium and free from sulphur. The fusion was made over an alcohol lamp.

Percentage composition of the ash-free antialbumid.

	I	II	III	IV	V	*Average*
C	53.59	53.45	....	....	....	53.52
H	6.86	6.83	....	....	....	6.84
N	....	....	13.56	13.74	....	13.65
S	....	....	....	....	2.22	2.22
O	....	....	....	....	....	23.77
						100.00

Comparison of the following figures shows the relationship in composition between the antialbumid and the mother substance from which it was derived.

	Coagulated egg-albumin.[1]	*Antialbumid.*
C	52.18	53.52
H	6.93	6.84
N	15.81	13.65
S	1.87	2.22
O	23.21	23.77

Most conspicuous is the marked loss of nitrogen and the corresponding rise in carbon; results which accord more or less closely with the earlier data obtained by Kühne and Chittenden.[2] Also noticeable is the somewhat larger percentage of sulphur present in antialbumid. A large amount of this sulphur exists loosely combined in the antialbumid molecule, and the question at once arises whether this loosely combined sulphur can be driven off by continued hydrolysis with dilute sulphuric acid. This point was tested by taking a portion of the antialbumid prepared as above and heating it further with 3 per cent sulphuric acid. After exposure at 100° C. in the sterilizer for 6 hours the acid fluid was found to yield a strong reaction for loosely combined sulphur, while the residue of antialbumid gave an equally marked reaction for loosely combined sulphur. The acid fluid was therefore filtered off, the antialbumid washed with water, and again heated for some hours with fresh 3 per cent sulphuric acid. This process was repeated until the aggregate period of heating had reached 38 hours, at the end of which time the last acid fluid was found free from loosely combined sulphur. The antialbumid remaining, however, still gave a striking reaction for sulphur with potassium hydroxide and plumbic acetate, thus showing

[1] Chittenden and Bolton: Studies in physiological chemistry, Yale University, 1887, ii, p. 130.

[2] Kühne and Chittenden: Zeitschrift für Biologie, 1883, xix, p. 176.

that the loosely combined sulphur contained in the antialbumid molecule cannot be removed entirely by this method of hydrolysis. Especially noteworthy was the continued shrinkage of the antialbumid during this long process of heating with the dilute acid, until at the termination of the treatment the substance was greatly diminished in bulk; a fact which accords with Schutzenberger's[1] original observations that this substance, when heated continuously with dilute acid, slowly but progressively disappears. Somewhat noticeable, however, is the fact that such portion of the antialbumid as does resist this long continued action of the dilute sulphuric acid is not widely different in composition from the original antialbumid. Thus, analysis of the product remaining after the 38 hours' heating gave the following results:

I. 0.1971 gram substance gave 0.1245 gram H_2O = 7.01 per cent H and 0.3937 gram CO_2 = 54.48 per cent C.
II. 0.3016 gram substance gave 0.6035 gram CO_2 = 54.58 per cent C.
III. 0.2972 gram substance gave by the Kjeldahl method 0.04113 gram N = 13.84 per cent N.
IV. 0.2093 gram substance gave 0.02860 gram N = 13.66 per cent N.
V. 0.5487 gram substance gave by fusion with NaOH and KNO_3 0.1058 gram $BaSO_4$ = 2.63 per cent S.
VI. 0.4770 gram substance gave 0.0015 gram ash = 0.31 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	*Average*
C	54.65	54.75	....	....	....	54.70
H	7.02	....	....	....	....	7.02
N	....	....	13.88	13.71	....	13.79
S	....	....	....	....	2.64	2.64
O	....	....	....	....	....	21.85
						100.00

From these data it is evident that the antialbumid which has resisted this long continued treatment with dilute sulphuric acid has gained somewhat in its content of sulphur and still more in its content of carbon. Nitrogen, on the contrary, remains essentially the same. This tendency of an antialbumid, on hydrolysis, to grow richer in carbon usually at the expense of the nitrogen is exceedingly characteristic. Thus, it was found by Kuhne and Chittenden[2] on subjecting a sample of antialbumid (from egg-albumin) to the action of an alkaline solution of trypsin that the antialbumid remaining undi-

[1] Schützenberger: Bulletin de la société chimique de Paris, 1875, xxiii, p. 161.
[2] Kühne and Chittenden: *loc. cit.*

gested contained 55.54 per cent of carbon when dry, while the original antialbumid contained 53.79 per cent of carbon. Similarly, an antialbumid, prepared from serum-albumin, containing 54.51 per cent of carbon and 14.31 per cent of nitrogen, on being subjected to the action of trypsin in an alkaline medium yielded an undigested residue containing 58.09 per cent of carbon and 12.61 per cent of nitrogen; results which clearly testify to the innate tendency of antialbumid under suitable conditions to undergo hydrolysis and presumably also cleavage with formation of a more insoluble and resistant body, of the antialbumid type, with a higher content of carbon.

With a view to the possible preparation of a melanin, 30 grams of pure dry antialbumid (the first sample analyzed) were placed in a flask with 300 c.c. of 10 per cent sulphuric acid, the flask connected with an inverted Liebig's condenser, and the mixture boiled directly over a flame for 79 hours. When first heated, the acid was almost colorless, but as decomposition progressed the color changed first to purple and finally to jet black. Early in the course of the heating a light film, shown later to be composed of fatty acids, was deposited on the sides of the condenser, and the odor of volatile fatty acids was very distinct.[1] As the boiling progressed, bright yellow crystals in rosettes were seen, both within the condenser and clinging to the neck and sides of the flask. These crystals were eventually collected and tested. They burned with a blue flame, forming acid fumes; they were insoluble in water, alcohol, and ether, but rapidly soluble in carbon disulphide. From the latter fluid they recrystallized in the characteristic rhombic octahedra of native sulphur.

At the conclusion of the 79 hours of heating, and after the acid fluid had cooled, the mixture was filtered through paper, leaving a residue of black amorphous matter, while the clear filtrate was quite black in color. Obviously, the fluid contained considerable black pigment in solution, but owing to the large admixture of leucin, tyrosin, and other substances present, attempts to isolate the pure pigment from the solution were not very successful. Attention was therefore directed to the insoluble pigment on the filter paper. This was washed with water until free from acid, and then with alcohol and ether, in all of which it was insoluble. As the pigment was found to be extremely soluble in weak alkalies, the precipitate was

[1] See R. Cohn: Zeitschr. f. physiol. Chem., 1896, xxii, p. 153.

treated with a very little 0.2 per cent potassium hydroxide solution, in which it quickly dissolved, leaving a small slate-colored residue (too small to identify) which was readily washed free from the melanin-like substance. Although the volume of the weak alkaline fluid with the washings now amounted to full 500 c.c. the clear fluid was jet black in color and absolutely opaque to light. On neutralization of this fluid with dilute acetic acid the melanin was reprecipitated in large dark flocks, leaving a colorless transparent fluid. The pigment was filtered off, washed entirely free from salts with water, and thoroughly extracted with alcohol and ether, after which it was dried to a constant weight at 105° C. When dry the preparation weighed 1.498 grams, equal to practically 5 per cent of the original antialbumid.

On analysis this substance gave the following results:

I. 0.3598 gram substance gave 0.2249 gram H_2O = 6.94 per cent H and 0.7158 gram CO_2 = 54.26 per cent C.

II. 0.2044 gram substance gave by the Kjeldahl method 0.02453 gram N = 12.00 per cent N.

III. 0.2273 gram substance gave 0.02733 gram N = 12.00 per cent N.

IV. 0.3691 gram substance gave by fusion with NaOH and KNO_3 0.2063 gram $BaSO_4$ = 7.70 per cent S.

Percentage composition of the melanin.[1]

	I	II	III	IV	*Average*
C	54.26	....	....	....	54.26
H	6.94	12.00	12.00	....	6.94
N	....	....	....	....	12.00
S	....	....	....	7.7	7.70

A second decomposition, using a larger amount of the same antialbumid — 61 grams — was now attempted under exactly the same conditions as before, except that the acid mixture was boiled for 110 hours. Fatty acids and free sulphur were detected as before, while the same separation of a black pigment took place. The latter was collected on two filters and the precipitates washed as already described, after which the larger precipitate was dissolved in water containing a little ammonia, the clear filtered solution precipitated by neutralization with 0.2 per cent hydrochloric acid, and the pre-

[1] The ash could not be determined with any accuracy owing to the scarcity of substance, but the small amount of ash left in the platinum tray in the determination of carbon made it quite clear that not more than a small fraction of one per cent could be present.

cipitate thoroughly washed with water, alcohol, and ether. When dry, it weighed 2.728 grams. The smaller portion of crude melanin was dissolved in 0.2 per cent solution of potassium hydroxide and the clear filtered solution precipitated with dilute hydrochloric acid, etc. as just described. When dry, this preparation weighed 0.675 gram. Hence, the total yield of melanin insoluble in the 10 per cent sulphuric acid amounted to 3.403 grams, or 5.5 per cent of the original antialbumid.

The melanin formed in this decomposition — the portion which had been purified by solution in dilute ammonia — gave on analysis the following results, after being dried at 105° C.

I. 0.3140 gram substance gave 0.2130 gram H_2O = 7.53 per cent H and 0.6631 gram CO_2 = 57.60 per cent C.

II. 0.2012 gram substance gave 0.1300 gram H_2O = 7.17 per cent H and 0.4267 gram CO_2 = 57.85 per cent C.

III. 0.2247 gram substance gave by the Kjeldahl method 0.02671 gram N = 11.88 per cent N.

IV. 0.2245 gram substance gave 0.02656 gram N = 11.83 per cent N.

V. 0.5479 gram substance gave by fusion with NaOH and KNO_3 0.1749 gram $BaSO_4$ = 4.39 per cent S.

VI. 0.4050 gram substance gave by fusion with NaOH and KNO_3 0.1258 gram $BaSO_4$ = 4.27 per cent S.

VII. 0.4616 gram substance gave 0.0025 gram ash = 0.54 per cent.

Percentage composition of the ash-free substance.

	I	II	III	IV	V	VI	*Average*
C	57.91	58.16	....	....	....	....	58.05
H	7.57	7.21	....	....	....	....	7.39
N	....	....	11.94	11.90	...	....	11.92
S	....	....	....	....	4.41	4.29	4.35
O	....	....	....	....	....	....	18.29
							100.00

The smaller fraction of this same melanin which had been purified by solution in 0.2 per cent potassium hydroxide contained the following percentages of nitrogen and sulphur:

0.2046 gram substance gave by the Kjeldahl method 0.02379 gram N = 11.63 per cent N.
0.4070 gram substance gave by fusion with NaOH and KNO_3 0.1272 gram $BaSO_4$ = 4.29 per cent S.

These results seemingly show that the sulphur and nitrogen content of the melanin is not materially affected by the character of the alkali used to dissolve it; *i. e.* a fixed alkali, when dilute, causes no marked withdrawal of sulphur or nitrogen. Natural pigment from

hair or epidermis when treated with 10 per cent solution of potassium hydroxide, and then reprecipitated by acid is liable to lose both nitrogen and sulphur,[1] but whether this is due to a change in the pigment itself produced by the strong alkali, or whether due to the withdrawal of contaminating substances derived from the original pigmentary granules, is uncertain.

Let us compare now the composition of our artificial melanins with that of the antialbumid from which they were derived.

	Antialbumid.	*Melanin.* 79 hours boiling.	*Melanin.* 110 hours boiling.	*Antialbumid.*[2]
C	53.52	54.26	58.05	54.70
H	6.84	6.94	7.39	7.02
N	13.65	12.00	11.92	13.79
S	2.22	7.70	4.35	2.64

The results seemingly justify the conclusion that these melanins formed from antialbumid originate not by simple hydrolysis, but by a process of hydrolytic cleavage, the pigment holding the position of a cleavage residue, the exact composition of which depends upon the extent or intensity of the cleavage process. Further, it is evident that this melanin-like residue, *i. e.* the true pigment, is either contaminated by some substance or substances, which accounts for the marked variation in composition, or else that under the term *melanins* we have a class of related bodies more or less alike in their physical properties but unlike in chemical composition. Certainly, our knowledge regarding the composition of the natural melanins lends favor to the latter view, for it is a well-known fact that certain melanins are exceedingly rich in sulphur (10 per cent), like the phymatorhusin of Berdez and Nencki,[3] while others, like the choroidal pigment,[4] are entirely free from sulphur. Again, while the majority of these pigments contain practically no iron at all, others contain 0.5 per cent of this element.[5] Hence there may be justification for the suggestion made by Brandl and Pfeiffer[6] that the melanins should

[1] See Abel and Davis: Journal of experimental medicine, 1896, i, p. 391. Also, M. Nencki and N. Sieber: Archiv f. exper. Pathol. u. Pharmakol., 1887, xxiv, p. 17.

[2] After 38 hours' heating with 3 per cent sulphuric acid.

[3] Berdez and M. Nencki: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.

[4] Sieber: *Ibid.*, 1886, xx, p. 362.

[5] See Brandl and Pfeiffer: Zeitschrift für Biologie, 1890, xxvi, p. 348.

[6] *Loc. cit.*

be divided into groups to be designated as ferro-melanins, sulpho-melanins, etc. The melanins which we have prepared from anti-albumid are practically free from iron, the ash containing only the merest trace of this element. Somewhat suggestive is the fact that in both of our experiments with antialbumid, in spite of the difference in the length of the hydrolytic process, the yield of melanin in both cases amounted to about 5 per cent.

Formation of melanin from so-called hemipeptone. — Having demonstrated the possibility of preparing a melanin-like substance by the hydrolysis of antialbumid, the question naturally arose whether bodies of the so-called hemi class will likewise yield a melanin by hydrolysis. To test this point, so-called hemipeptone formed by the hydrolysis of coagulated egg-albumin with 3 per cent sulphuric acid was employed. The peptone was prepared by using the acid fluid, resulting in the formation of antialbumid. The fluid was neutralized with ammonia, the filtered solution concentrated and the albumoses separated collectively by saturation of the fluid with ammonium sulphate, after the method suggested by Kühne.[1] After complete removal of the albumoses, the excess of ammonium sulphate was separated by alternate concentration and crystallization, after which the last portions of the salt were removed by treatment with barium carbonate.[2] The filtrate, freed from all traces of barium by cautious addition of dilute sulphuric acid, was concentrated to a syrup and the peptone precipitated by alcohol. The product was purified somewhat by repeated boiling with alcohol and thorough extraction with ether.

In the production of a melanin 90 grams of this hemipeptone were boiled for 98 hours with 2 litres of 10 per cent sulphuric acid. The fluid rapidly became black in color, and gradually there occurred a small separation of a black pigment. There was also noticeable, especially toward the end of the boiling, the odor of volatile fatty acids, and some slight crystallization of fatty acids upon the walls of the condenser could be detected likewise. There was however no evidence of the splitting off of free sulphur, so conspicuous with anti-albumid. At the expiration of the 98 hours, the fluid was cooled, and the melanin-like substance collected by filtration. The very dark filtrate on standing and concentrating continued to deposit

[1] KÜHNE: Zeitschrift für Biologie, 1892, xxix, p. 1.

[2] See CHITTENDEN, MENDEL, and HENDERSON: This journal, 1899, ii, p. 173.

small portions of black pigment, the process being repeated until several fractions were obtained. The fluid, however, still gave evidence of the presence of considerable pigment in solution. The united fractions of pigment were washed thoroughly with water, then dissolved in 0.2 per cent solution of potassium hydroxide and the filtered fluid precipitated by neutralization with 0.2 per cent hydrochloric acid. The pigment was next washed free from soluble salts with water and extracted with alcohol and ether. When freshly precipitated this melanin, unlike that prepared from antialbumid, was somewhat soluble in water, but if allowed to dry upon the filter it could be washed with water without loss. The amount of pigment obtained was 0.9 gram, equal to 1.0 per cent of the original hemipeptone.

When dried at 105° C. until of constant weight, it gave on analysis the following results:

I. 0.2435 gram substance gave 0.0815 gram H_2O = 3.72 per cent H and 0.5156 gram CO_2 = 57.75 per cent C.

II. 0.2068 gram substance gave by the Kjeldahl method 0.01986 gram N = 9.60 per cent N.

III. 0.2796 gram substance gave by fusion with NaOH and KNO_3 0.0569 gram $BaSO_4$ = 2.79 per cent S.

IV. 0.2435 gram substance gave 0.0192 gram ash = 6.11 per cent.

Percentage composition of the ash-free substance.

	I	II	III	*Average*
C	61.50	....	....	61.50
H	3.97	....	....	3.97
N	....	10.23	....	10.23
S	....	....	2.98	2.98
O	....	....	....	21.32
				100.00

It is thus plainly evident that the melanin obtained from hemipeptone is widely different in chemical composition from the corresponding body obtained from antialbumid. It resembles in its content of carbon the melanin-like body prepared by Schmiedeberg from Witte's "pepton," but differs widely from it in the amount of both nitrogen and sulphur. It would seem that the artificial melanins differ as widely among themselves in composition as the natural melanins obtainable from melanotic tumors, etc., or from normal pigmentary deposits. Certainly the contrast between the melanoidic acid with its 66 per cent of carbon obtained by Schmiedeberg from serum-albumin and the corresponding pigments with 54, 58, and 61 per

cent of carbon respectively obtained by us from antialbumid and hemipeptone, is very striking.

Reactions of the melanins. — The pigments formed in the manner described above were practically insoluble in water, although, as stated, the sample obtained from hemipeptone was somewhat soluble when freshly precipitated. They were likewise insoluble in alcohol, ether, chloroform, etc., but readily soluble in exceedingly dilute alkaline fluids. They thus differ in some slight degree from the pigment isolated by Abel and Davis[1] from the negro's skin. Further, these pigments seemingly retain their solubility in dilute alkalies in greater degree than the pigments described by Abel and Davis or the melanoidic acid prepared by Schmiedeberg[2] from serum-albumin.

When dry, the pigments were more or less jet black in color, and when dissolved in dilute alkali they yielded yellowish brown, brown, or black colored solutions according to the degree of concentration. These solutions show no absorption bands when examined before the spectroscope, but absorb a certain amount of light at the violet end of the spectrum. Unlike the melanin obtainable from the negro's skin,[3] our pigments were soluble in glacial acetic acid, but were not precipitable therefrom by potassium ferrocyanide. Alkaline solutions of these artificial melanins were completely bleached by chlorine.

Heated on platinum foil, our products, like the pigments described by Abel and Davis, gave off at first fumes of pyrrol, — tested by a pine-sliver moistened with hydrochloric acid, — but these soon ceased, leaving a coal-black residue very difficult of combustion.

From alkaline solutions, the pigments were precipitated by cupric sulphate, silver nitrate, plumbic acetate, and baryta water. By strong nitric acid the pigments were dissolved, but precipitated again on addition of water.

From these few reactions it is seen that our artificial melanins or melanoidins, to use Schmiedeberg's term, differ only in minor degree from the melanins or pigments obtained by Abel and Davis from the skin and hair of the negro, or from the melanins studied by other observers.

Comparison of the composition of the artificial melanins with that of natural melanins, etc. — Our own results bearing on the chemical

[1] Abel and Davis: Journal of experimental medicine, 1896, i, p. 386.

[2] Schmiedeberg: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxix, p. 66.

[3] Abel and Davis: *loc. cit.*

Origin of the Pigment.	Author.	Percentage Composition.				
		C	H	N	S	Fe
Hydrolysis of serum-albumin	Schmiedeberg [1]	66.27	5.49	5.57		
Hydrolysis of Witte's "pepton"	Schmiedeberg [1]	60.34	4.86	8.09	0.96	
Hydrolysis of Antialbumid [2]	Chittenden and Albro	54.26	6.94	12.00	7.70	
Hydrolysis of Antialbumid [3]	Chittenden and Albro	58.05	7.39	11.92	4.35	
Hydrolysis of Hemipeptone [4]	Chittenden and Albro	61.50	3.97	10.23	2.98	
Sarcoma (human) of liver and spleen	Berdez and Nencki [5]	53.58	4.22	10.59	10.13	
Sarcoma of horse, liver and spleen	Berdez and Nencki [6]	53.52	3.92	10.48	2.78	
Human urine, sarcoma	K. A. H. Mörner [7]	55.76	5.95	12.27	9.01	0.2
Tumor, horses' spleen	Miura [8]	54.50	5.06	11.75	2.72	
Human liver, sarcoma	Brandl and Pfeiffer [9]	53.58	4.00	9.91	3.02	0.53
Ox eyes, choroidal pigment	Sieber [10]	59.90	4.61	10.81	0	0
Black and brown hair	Sieber [10]	56.14	7.57	8.50	4.10	
Epidermis of negro	Abel and Davis [11]	53.56	5.11	15.47	2.53	
Hair of negro	Abel and Davis [11]	57.06	5.45	12.87	1.77	

[1] Schmiedeberg: Archiv f. exper. Pathol. u. Pharmakol., 1897, xxxix, p. 67.

[2] 79 hours' boiling with sulphuric acid.

[3] 110 " " "

[4] 98 " " "

[5] Berdez and M. Nencki: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 346.

[6] Berdez and M. Nencki: *Ibid.*, 1887, xxiv, p. 17.

[7] K. A. H. Mörner: Zeitschr. f. physiol. Chem., 1887, xi, p. 66.

[8] Miura: Virchow's Archiv fur pathologische Anatomie, 1887, cvii, p. 250.

[9] Brandl and Pfeiffer: Zeitschrift fur Biologie, 1896, xxxvi, p. 348.

[10] N. Sieber: Archiv f. exper. Pathol. u. Pharmakol., 1886, xx, p. 362.

[11] Abel and Davis: Journal of experimental medicine, 1896, i, p. 302.

composition of the melanin-like pigments obtainable by decomposition of proteids emphasize the view that these substances are many in number, and that, while having many points in common, they differ widely from each other in composition, owing no doubt in part to variations in the extent or intensity of the hydrolytic cleavage by which they are produced. This we fancy is a far more potent factor than the character of the individual proteid from which they are derived. Thus, in our experiments with antialbumid, the first preparation of melanin resulting from 79 hours' boiling of the proteid with sulphuric acid contained 54 per cent of carbon, while the second preparation resulting from 110 hours' boiling contained 58 per cent of carbon. Further, in the content of sulphur the differences were still more striking. The table on page 303, showing the composition of melanins from various sources, affords evidence of the extent to which these black pigments may differ from each other in chemical composition as well as indicating the extent to which the artificial melanins resemble the natural pigments.

These figures, which are fairly typical of the composition of the various melanins hitherto studied, show quite clearly how widely these bodies may vary in composition; yet throughout the entire list, as well as in many other preparations not tabulated, there is to be found almost invariably a relatively high content of carbon and a low content of nitrogen. Further, in all but the choroidal pigment, sulphur is very conspicuous. These facts, coupled with the inappreciable amounts of iron usually found, strengthen belief in the theory that these pigments, whether formed normally or as a result of pathological conditions, have their origin not in the hæmatin molecule but in proteid matter. Further, we see in the experiments of Schmiedeberg, as well as in our own results, evidence that serum-albumin, proteoses, antialbumid, and hemipeptone may all yield melanin-like substances by simple hydrolytic cleavage: pigments which in composition and reaction differ from the natural pigments no more widely than the latter differ from each other. No two melanins are exactly alike in composition, and the artificial bodies, certainly, are exceedingly prone to vary in composition with variations in the method of preparation; especially, variations in the extent and intensity of the hydrolytic cleavage. In the hydrolytic cleavage induced by boiling acids proteid substances tend to lose nitrogen, partially as ammonia and partially in the form of amido-acids and nitrogenous bases, while the artificial melanins simultaneously formed appear to

have their origin in the carbon-rich residue left after the splitting off of these nitrogenous radicles. For this reason, perhaps, antialbumid is especially well adapted to be the mother substance of a melanin. Between antialbumid and the melanins there is a certain recognizable kinship in composition, which renders the formation of a melanin-like pigment from this peculiar form of proteid matter an easy task. It is equally clear, from our experiments, however, that melanin may be formed likewise from such dissimilar proteid substances as so-called hemipeptone, from which we are forced to the conclusion that no one form of proteid matter is the sole antecedent of these peculiar brownish black pigments.

A NOTE ON THE CHOLESTERIN-ESTERS OF BIRDS' BLOOD.

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THE interesting investigations of Hürthle[1] have demonstrated that cholesterin occurs normally in combination with fatty acids in the blood-serum of mammals. It has been pointed out that the failure to recognize these cholesterin-esters has been due to the methods employed in the search for cholesterin. Thus the customary procedure has been to saponify ether extracts of animal tissues and fluids with alcoholic potash for the purpose of transforming any fats present into soaps and in this way permitting a more successful subsequent separation of cholesterin by means of ether.[2] The method obviously precludes the possibility of obtaining cholesterin-esters as such. By avoiding the saponification, however, Hürthle has succeeded in demonstrating the presence of cholesteryl oleate in the blood-serum of the dog, sheep, pig, ox, and horse, and in the lymph obtained from the thoracic duct of the dog. Cholesteryl palmitate was also obtained, although in much smaller quantities; while cholesteryl stearate could not be isolated. The quantities of the cholesterin-esters present in the blood were approximately determined as follows:

	Cholesteryl oleate	*Cholesteryl palmitate*
Horse	0.08 per cent	0.006 per cent
Calf	0.09 "	0.008 "
Dog	0.12–0.22 "	

The percentage of cholesteryl oleate was observed to vary in the dog with the condition of the animal, being increased during hunger.[3]

In his contributions to the chemistry of the blood, Hoppe-Seyler[4] has recorded analyses of the blood of the goose which indicate that cholesterin is present in the serum of this species in quantities approaching those of the ox.

[1] Hürthle: Zeitschrift f. physiol. Chemie, 1896, xxi, p. 331.

[2] Cf. Hoppe-Seyler: Medicinisch-chemische Untersuchungen, 1866, p. 143.

[3] Cf. Hoppe-Seyler: *loc. cit.*, p. 145; Schulz, Fr. N.: Arch. f. d. ges. Physiol., 1896, lxv, p. 299.

[4] Hoppe-Seyler: *loc. cit.*, p. 145.

In the course of some experiments in this laboratory, on the chemistry of birds' blood, the blood-serum of the hen, turkey, goose, and duck has been examined for cholesterin-esters. The observations made are recorded briefly here, since they verify and extend the investigations of Hurthle, whose methods have been employed, for the most part, in the preparation of the esters. The blood-serum was usually precipated with three volumes of alcohol; after standing, the precipitate was filtered off and extracted with fresh alcohol for two or three days at 40° C. Good results were obtained by keeping the precipitate continually agitated in the warm alcohol by means of a slow current of air. The filtered extract deposited the characteristic small needle crystals of the oleate on standing in the cold, while the surface of the alcohol was usually covered with a slight film having a more or less crystalline character (cholesteryl palmitate). The film was separated as far as possible, and the larger mass of fine needle crystals, often grouped in rosettes, was filtered off, washed with cold alcohol, and dried in vacuo over sulphuric acid. The crystals thus obtained were weighed, in order to afford an approximate idea of the quantity of material obtainable from the various serums; this method, as Hurthle has observed, by no means gives accurate determinations, inasmuch as quite appreciable quantities of the corresponding ester remain in solution in the alcohol used. The precipitated serum residues were usually re-extracted with warm alcohol, and finally treated with alcohol-ether for the separation of the remaining cholesteryl palmitate. The yield of substance in the latter process was always small. The ester preparations obtained were purified by recrystallization from alcohol, until the melting points corresponded with those found by Hürthle. In some cases the composition of the product was further established by an elementary analysis. The substances isolated all gave the cholesterin-like reactions described for them.

Hen serum. — Several preparations of cholesteryl oleate were separated from the serum of hens' blood and purified. They all melted at 43–44° C. An analysis of two products gave the following results:

Analysis of Cholesteryl oleate

	Preparation I	Preparation II	Hurthle's average	$C_{44}H_{76}O_2$: Theory
	Per cent	Per cent	Per cent	Per cent
Carbon . .	82.68	82.49	82.84	83.02
Hydrogen .	12.04	11.90	11.77	11.95

The approximate yield of cholesteryl oleate did not vary greatly. Thus:

I.	500 c.c. serum yielded	0.24 gram	= 0.05	per cent
II.	610 c.c. "	0.19 "	= 0.03	"
III.	1660 c.c. "	0.78 "	= 0.05	"
IV.	2200 c.c. "	1.05 "	= 0.05	"

From over four litres of serum about 0.2 gram substance was obtained, having a melting point (after recrystallization) of 77–78° C. and thus corresponding with the cholesteryl palmitate. The crystalline form also confirmed this deduction.

Turkey serum. — In one instance a relatively large yield of cholesteryl oleate was obtained from turkey serum. Thus:

I.	600 c.c. serum yielded	0.37 gram	= 0.06	per cent
II.	1000 c.c. "	1.43 "	= 0.14	"

The preparations melted at 43–44° C. and one of them (II) showed the following composition on analysis:

Carbon	82.84 per cent
Hydrogen	11.79 "

A preparation corresponding in crystalline form and solubilities with cholesteryl palmitate melted (after recrystallization) at 77–78° C.

Goose serum. — From 220 c.c. of this serum about 150 mgr. (0.07 per cent) of characteristic rosettes of needles were obtained; they melted after recrystallization at 43–44° C., thus corresponding to cholesteryl oleate.

Duck serum. — Considerable difficulty was experienced in obtaining characteristic cholesteryl oleate preparations from duck serum. The products showed admixture of apparently amorphous material, and melted at about 60° C. From one half litre serum, however, about 0.4 gram (0.08 per cent) of crystals was obtained. After recrystallization, the crystals melted at 42–44° C. By extracting the serum residue with alcohol-ether, one half gram of a crystalline product was obtained. The preparation, when purified, melted at 76 °C., giving evidence of the probable presence of a considerable quantity of cholesteryl palmitate (melting, when pure, at 77° C.).

The blood-corpuscles. — That cholesterin may occur in an uncombined state in the blood-corpuscles seems probable from the recent analyses of Abderhalden,[1] who found noticeable quantities of choles-

[1] Abderhalden: Zeitschrift f. physiol. Chemie, 1897, xxiii, p. 522; *Ibid.*, 1898, xxv, p. 108.

terin in them, while fatty acids were absent, or present only in very small amounts. The older statements of Hoppe-Seyler[1] lead to similar conclusions; and Wooldridge[2] stated that he obtained cholesterin free from fats and lecithin by extracting the stroma of blood-corpuscles with cold ether. In the present experiments crystals of cholesterin were repeatedly obtained from the blood-corpuscles by direct extraction with ether. The corpuscles were separated from defibrinated blood by centrifugalization or by subsidence, and after treatment once or twice with one per cent sodium chloride solution to remove any adherent serum, were rendered laky and extracted. The ether extract corresponded in its behavior with the description given by Wooldridge; on evaporation it yielded a residue of needle-shaped crystals occasionally arranged in rosettes. After recrystallization from alcohol-ether the more characteristic rhombic tables appeared. These crystals showed the characteristic color reaction with chloroform and concentrated sulphuric acid, and the absence of fats or fatty acids was demonstrated by the fact that they melted at a temperature above 100° C. The corpuscles of the sheep, dog, hen, and turkey were examined, and cholesterin crystals obtained in every instance.[3]

I desire to acknowledge the kind advice of Professor Lafayette B. Mendel, at whose suggestion these experiments were carried out.

[1] Hoppe-Seyler and Thierfelder: Handbuch der chemischen Analyse für Aerzte, p. 408.

[2] Wooldridge: Archiv für Physiologie, 1881, p. 389.

[3] Since the preceding account was sent for publication, a paper by E. Hepner: "Ueber den Cholestearingehalt der Blutkörperchen," has appeared in the Archiv f. d. ges. Physiol., 1899, lxxiii, p. 595. The cholesterin-content of the corpuscles of the dog and horse was determined; free cholesterin was also detected in the blood-plasma.